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# NET ENERGY VALUES OF FEEDING STUFFS FOR CATTLE


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## INTRODUCTION

Besides supplying certain specific forms of matter (ash ingredients, proteins, lipoids, carbohydrates, vitamines, etc.) essential to the normal course of metabolism, the feed of an animal is, so far as we know, the sole source of the energy whose transformations constitute the essential phenomena of physical life. This energy is contained in the feed as chemical energy, and the maximum quantity which any substance can furnish for the vital activities by its oxidation in the body is measured by its heat of combustion. It rarely, if ever, happens, however, that this maximum effect is realized. In practically every case a larger or smaller proportion of the chemical energy of the feed escapes unutilized. These losses of energy are of two general classes.

First, a portion of the chemical energy of the feed fails to be transformed at all, leaving the body as chemical energy in the visible excreta and in the combustible gases arising from gastric and intestinal fermentations.

Second, another portion of the chemical energy of the feed is indeed transformed, but at ordinary temperatures virtually results merely in a superfluous heat production. It is true that the metabolism consequent upon feed consumption is not only unavoidable but may be regarded as a necessary expenditure of energy for the support of the activities connected with digestion and assimilation. Nevertheless, from the standpoint of the net gain or loss by the organism this portion of the feed energy, which ultimately takes the form of heat and escapes from the body, must be regarded as a loss.

The remainder of the chemical energy in the feed, after deducting these two classes of losses, has been designated as its net energy value and

expresses the net effect of the feed either in causing a storage of chemical energy in the form of fat, protein, etc., in the body, or, in the case of a submaintenance ration, in diminishing the amount of energy which must be supplied by the katabolism of body tissue.

The investigations of the last 30 years have shown that both the general problems of nutrition and the economic questions relating to the feeding of domestic animals may be advantageously studied from the standpoint of energetics. From this standpoint, it is of importance to determine as accurately as may be the losses of energy which feed substances undergo in the two ways just mentioned and the resulting net energy values. In the following pages are reported the results of a considerable number of experiments on cattle carried out at this Institute during the years 1902 to 1912, inclusive, in which these losses have been determined for certain feeding stuffs.[1] These experiments up to the end of 1907 have been already reported in full (7, 8, 9, 10) [2] and it is hoped to discuss the details of the later ones in subsequent papers. Here it will be convenient, following a general description of the experiments, to consider:

I. The losses of chemical energy.
II. The expenditure of energy consequent upon feed consumption and its factors.
III. Net energy values and their computation.

## GENERAL DESCRIPTION OF THE EXPERIMENTS

The experiments were made with the aid of a respiration calorimeter of the Atwater-Rosa type, the essential features of which have already been described (3, 4, 7). The apparatus permits a determination of the water vapor and carbon dioxid excreted, of the carbon and hydrogen in the combustible gases produced, and of the heat given off, but not of the oxygen consumed. In addition to the ordinary feeding stuffs analyses of feed and excreta, the quantitative collection of the feces and urine and the determination of the amounts of carbon, hydrogen, and energy contained in them were also necessarily involved. The experiments comprised, in all, 76 single feeding periods. Each period covered at least 3 weeks, of which 11 days or more constituted a preliminary period, while the visible excreta were collected for the last 10 days, during which, on the seventh and eighth days, the complete balance of matter and energy was determined for 48 consecutive hours in the respiration calorimeter.[3] The accuracy of this instrument was tested by means of numerous alcohol checks. The results of 18 such checks (10, p. 217–222) showed that the

[1] In all, over 30 persons have taken a more or less direct part in the respiration trials and in the large amount of analytical, clerical, and miscellaneous work involved in the experiments. For obvious reasons, it is impossible even to attempt any statement of the exact part taken by individuals or to make acknowledgments for the specific work done by each person. This is all the more true because the most important factor in whatever success the investigation has attained, and one which by its nature is incapable of such partition, is the loyalty and zeal which all concerned have shown in the execution of the plan of the investigation and in securing the greatest attainable accuracy of details.

[2] Reference is made by number to "Literature cited," p. 489–491.

[3] For details regarding the methods employed compare the bulletins of the Bureau of Animal Industry already cited (7, 8, 9, 10), especially Bulletin 128 (10), p. 200–216, as well as the detailed descriptions of the single experiments contained in that bulletin.

values obtained in a single experiment may be regarded as accurate to within the following percentages of the amounts determined: Carbon dioxid, 0.5; water, 6.0; heat, 1.0.

A further test of the accuracy of the work is found in a comparison of the observed heat production with that computed in the ordinary way from the balance of carbon and nitrogen. Comparisons of this sort for 57 feeding periods up to the end of 1909 (5, 6) showed an average difference of 0.4 per cent. The results of the later comparisons reduce this difference to 0.3 per cent, or, if the unsatisfactory results of the year 1905 be omitted, to 0.04 per cent. The total amounts of heat involved are as follows:

| | 76 periods. | 68 periods. |
|---|---|---|
| Computed heat production ........ Calories.. | 1,338,887 | 1,231,711 |
| Observed heat production ........ do.... | 1,343,071 | 1,231,251 |
| Difference ........ do.... | 4,184 | 460 |
| Percentage difference........ | 0.31 | 0.04 |

While the basis for the computation of the heat production is not altogether satisfactory, especially in the absence of determinations of the gain or loss of glycogen by the animal, nevertheless the general agreement is such as apparently to preclude the existence of gross errors.

## FEEDING STUFFS

The dry matter of the several feeding stuffs used had the following average composition, as shown in each case by concordant analyses of two or more separate samples taken at the beginning of each experiment (Table I). The more important determinations were also repeated upon samples taken when the feed was weighed out for each period, and these period results form the basis of the computations on subsequent pages.

TABLE I.—*Composition of the dry matter of the feeding stuffs*

| Feeding stuff and experiment No. | Ash. | Protein. | Non-protein. | Crude fiber. | Nitrogen-free extract. | Ether extract. | Heat of combustion per kilogram. |
|---|---|---|---|---|---|---|---|
| Timothy hay: | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Calories.* |
| 174........ | 4.64 | 5.11 | 0.24 | 38.92 | 49.01 | 2.08 | 4,554 |
| 190........ | 4.87 | 5.08 | .39 | 38.15 | 49.51 | 2.01 | 4,431 |
| 200........ | 5.86 | 6.72 | .83 | 33.02 | 51.01 | 2.56 | 4,516 |
| 207........ | 5.01 | 6.90 | .24 | 31.15 | 54.55 | 2.15 | 4,505 |
| Red clover hay: | | | | | | | |
| 179........ | 6.40 | 12.90 | 1.61 | 31.61 | 44.81 | 2.67 | 4,457 |
| 186........ | 6.57 | 11.18 | .89 | 28.78 | 49.65 | 2.93 | 4,492 |
| Mixed hay: | | | | | | | |
| 211........ | 7.01 | 9.62 | 1.29 | 33.78 | 46.00 | 2.30 | 4,396 |
| Alfalfa hay: | | | | | | | |
| 208........ | 9.40 | 11.86 | 2.52 | 31.06 | 42.75 | 1.51 | 4,403 |
| 209........ | 10.76 | 12.09 | 1.67 | 31.70 | 41.86 | 1.92 | 4,330 |
| 212........ | 9.06 | 12.39 | 2.86 | 30.10 | 43.63 | 1.96 | 4,368 |
| Alfalfa meal: | | | | | | | |
| 212........ | 9.24 | 11.75 | 2.87 | 31.12 | 43.17 | 1.85 | 4,374 |
| Maize stover: | | | | | | | |
| 210, total........ | 6.14 | 4.09 | .86 | 36.15 | 51.46 | 1.30 | 4,337 |
| 210, portion eaten | 6.67 | 4.46 | .98 | 35.23 | 51.25 | 1.41 | 4,332 |

TABLE I.—*Composition of the dry matter of the feeding stuffs*—Continued

| Feeding stuff and experiment No. | Ash. | Protein. | Non-protein. | Crude fiber. | Nitrogen-free extract. | Ether extract. | Heat of combustion per kilogram. |
|---|---|---|---|---|---|---|---|
| | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Calories.* |
| Maize meal: | | | | | | | |
| 179 | 1.37 | 9.94 | .48 | 2.60 | 81.38 | 4.23 | 4,431 |
| 211 | 1.62 | 9.29 | .24 | 3.16 | 80.64 | 5.05 | 4,517 |
| Wheat bran: | | | | | | | |
| 190 | 7.52 | 14.50 | .49 | 11.49 | 61.88 | 4.12 | 4,532 |
| Grain mixture No. 1:[a] | | | | | | | |
| 200 | 4.10 | 16.97 | 3.43 | 6.09 | 62.89 | 6.52 | 4,690 |
| 207 | 4.14 | 17.95 | 2.22 | 6.16 | 64.38 | 5.15 | 4,670 |
| Grain mixture No. 2:[b] | | | | | | | |
| 208 | 2.78 | 12.07 | .78 | 5.63 | 73.65 | 5.09 | 4,604 |
| 209 | 2.54 | 13.21 | .54 | 5.81 | 72.77 | 5.13 | 4,617 |
| Hominy chop: | | | | | | | |
| 211 | 2.75 | 9.33 | 1.29 | 5.13 | 72.65 | 8.85 | 4,709 |

[a] Wheat bran, 14.28 per cent; maize meal, 42.86 per cent; old process linseed meal, 42.86 per cent.
[b] Maize meal, 60 per cent; crushed oats, 30 per cent; old process linseed meal, 10 per cent.

## ANIMALS

Nine different steers have been used, varying in age from 11 months to approximately 60 months at the beginning of the several experiments.[1] They were either full bloods or high grades of recognized beef breeds, with one exception, steer B, which was distinctly of the dairy type and of mixed breeding (scrub), Jersey blood apparently predominating. All were docile animals and were thoroughly accustomed to the necessary handling, to wearing the apparatus for the collection of excreta, and to their surroundings in the digestion stall and the calorimeter. Further particulars concerning them are contained in Table II.

TABLE II.—*Description of the animals used in the experiments*

| Animal No. | Breed of animal. | Nos. of experiments in which used. | Age at beginning of each experiment. | Average live weight in each experiment. |
|---|---|---|---|---|
| | | | *Months.* | *Kilograms.* |
| I | Grade Shorthorn | 174 | 36 | 408 |
| | | 179 | 48 | 528 |
| | | 186 | 60 | 572 |
| A | Aberdeen Angus | 190 | 11 | 274 |
| | | 200 | 23 | 408 |
| | | 207 | 35 | 510 |
| B | Scrub | 190 | 13 | 195 |
| | | 200 | 25 | 303 |
| | | 207 | 37 | 380 |
| C | Grade Hereford | 208 | 9 | 275 |
| D | do | 208 | 9 | 167 |
| | | 210 | 21 | 331 |
| | | 211 | 33 | 449 |
| E | do | 208 | 9 | 208 |
| F | do | 209 | 21 | 300 |
| G | Full-blood Hereford | 211 | 28 | 379 |
| H | Full-blood Shorthorn | 212 | 20 | 345 |

[1] The word "experiment" is here used to designate the work of an entire season, including several feeding periods.

## I. LOSSES OF CHEMICAL ENERGY—METABOLIZABLE ENERGY

The losses of chemical energy occur substantially in the feces and urine and in the combustible gases.

The energy content of the dried feces and urine is readily determined. In investigations at this Institute, Braman (16) has shown that the loss of energy in the drying of urine may be estimated with a good degree of accuracy, the error being insignificant in comparison with the total energy of the feed. The possible loss of energy in the drying of the feces has not yet been investigated directly, although Fingerling, Köhler, and Reinhardt (18) have observed a loss of carbon, the amount of which they do not state.

The energy content of the combustible gases is not susceptible of direct determination, but must be estimated from their chemical composition. The combustible gases which have been actually identified as excreted by cattle are methane and hydrogen. All investigations are in accord in showing that the former is the chief product of the normal fermentations occurring in the digestive tract, but results differ regarding the extent to which hydrogen is formed. In our experiments the gases were analyzed by passing them over platinized kaolin at a red heat. By this method in almost every instance a ratio of C to H slightly greater than that in $CH_4$ (2.976 to 1) has been found, the average of 57 experiments reported by the junior author elsewhere (19) being 3.167 to 1, with considerable variations in individual cases. We are inclined to think that this high figure is due to failure to oxidize the last traces of hydrogen in the combustion tube. On the other hand, Markoff (37, 38), in his extensive investigations of paunch fermentation in cattle, found in nearly every instance a small amount of hydrogen, and Von der Heide, Klein, and Zuntz (20), in respiration experiments upon an ox, observed a small excretion of hydrogen in two cases out of four. In computing the energy losses in the following experiments it has been assumed that the combustible gases consisted of $CH_4$ (methane), and the computations have been based upon the observed quantity of carbon.

The difference between the chemical energy of the feed and that lost in the excreta shows how much of the former is capable of being converted into other forms in the body, either during the changes which the feed undergoes in the digestive tract or in the course of metabolism in the tissues. This convertible portion of the feed energy has been given various names by different investigators, such as "physiological heat value," "fuel value," "available energy," etc. Without entering here into a discussion of the propriety of these names, we have preferred for our present purpose to follow the suggestion made earlier by the senior author (2, p. 270) and to designate it as "metabolizable energy." By this term is meant simply the energy capable of transformation in the body, with no implications as to the proportion of the energy thus trans-

formed which can be utilized by the organism. The heat evolved during the methane fermentation, for example, constitutes part of the metabolizable energy as thus defined, although it does not enter into the tissue metabolism.

The determination of the losses of chemical energy from a single feeding stuff or from a mixed ration is relatively simple, as is illustrated by the following example taken from the results on steer B in experiment 207.

*Computation of losses of chemical energy from a ration*

| | Period 2. | | Period 3. | |
|---|---|---|---|---|
| Energy of feed: | | | | |
| Timothy hay | 12,477 Cals. | | 12,618 Cals. | |
| Grain mixture No. 1 | 12,549 Cals. | | ...... | |
| Total | | 25,026 Cals. | | 12,618 Cals. |
| Energy of excreta: | | | | |
| Feces | 7,371 Cals. | | 5,247 Cals. | |
| Urine | 1,536 Cals. | | 627 Cals. | |
| Methane | 2,098 Cals. | | 1,057 Cals. | |
| Total | | 11,005 Cals. | | 6,931 Cals. |
| Metabolizable energy | | 14,021 Cals. | | 5,687 Cals. |

Since concentrates can not be fed alone, the losses of chemical energy which they suffer, like their digestibility, must be obtained by means of a calculation by difference, which in period 2 of the foregoing example is as follows:

*Computation of losses of chemical energy by a concentrate*

| | Chemical energy of feed. Calories. | Chemical energy of excreta. Feces. Calories. | Urine. Calories. | Methane. Calories. | Metabolizable energy. Calories. |
|---|---|---|---|---|---|
| Total ration | 25,026 | 7,371 | 1,536 | 2,098 | 14,021 |
| Computed for hay | 12,477 | 5,254 | 591 | 1,003 | 5,629 |
| Grain mixture by difference | 12,549 | 2,117 | 945 | 1,095 | 8,392 |

Computed in the manner just illustrated, the losses of chemical energy per kilogram of dry matter consumed in these experiments and the metabolizable energy remaining are shown in Table III, which includes also the percentage distribution of the feed energy between the various excreta, on the one hand, and the metabolizable energy, on the other.[1] For convenience, the average results for the metabolizable energy per kilogram of dry matter and per kilogram of digestible organic matter are brought together in Table IV.

[1] In all cases the observed energy of the urine has been corrected to nitrogen equilibrium of the animal by adding 7.5 Calories for each gram of nitrogen retained by the animal or subtracting the same amount for each gram of body nitrogen lost, the correction being regarded as representing energy of excretory material temporarily retained in the body.

TABLE III.—*Losses of energy and their percentage distribution*

| Feeding stuff and experiment No. | Animal No. | Period No. | Dry matter eaten per day and head. | | Energy per kilogram of dry matter. | | | | | Metabolizable per kilogram of digestible organic matter. | Percentage losses. | | | Percentage metabolizable. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Losses. | | | | | | | | |
| | | | Coarse feed. | Concentrates. | Total.[a] | In feces. | In urine.[b] | In $CH_4$. | Metabolizable. | | In feces. | In urine. | In $CH_4$. | |
| Timothy hay: | | | *Gm.* | *Gm.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | | | | |
| 174[c] | I | D-A | 4,483 | 356 | 4,554 | 2,439 | 156 | 285 | 1,674 | 3.483 | 53.56 | 3.44 | 6.25 | 36.75 |
| 190 | A | 3 | 2,001 | ...... | 4,502 | 2,209 | 164 | 309 | 1,820 | 3.429 | 49.06 | 3.64 | 6.87 | 40.43 |
| 190 | A | 4 | 3,493 | ...... | 4,488 | 2,306 | 128 | 303 | 1,751 | 3.480 | 51.38 | 2.86 | 6.75 | 39.01 |
| 190 | B | 3 | 1,774 | ...... | 4,503 | 2,209 | 141 | 342 | 1,811 | 3.399 | 49.07 | 3.14 | 7.60 | 40.20 |
| 190 | B | 4 | 2,610 | ...... | 4,483 | 2,176 | 125 | 304 | 1,878 | 3.484 | 48.55 | 2.79 | 6.78 | 41.88 |
| Average | | | 2,470 | ...... | 4,494 | 2,225 | 139 | 315 | 1,815 | 3,448 | 49.51 | 3.09 | 7.01 | 40.39 |
| 200 | A | 3 | 2,647 | ...... | 4,510 | 2,175 | 155 | [b]314 | 1,866 | 3.597 | 48.22 | 3.44 | 6.97 | 41.37 |
| 200 | A | 4 | 4,425 | ...... | 4,509 | 2,250 | 153 | [b]301 | 1,805 | 3.566 | 49.91 | 3.40 | 6.68 | 40.01 |
| 200 | B | 3 | 2,470 | ...... | 4,510 | 2,092 | 168 | [b]326 | 1,924 | 3.565 | 46.39 | 3.72 | 7.24 | 42.65 |
| 200 | B | 4 | 3,805 | ...... | 4,509 | 2,164 | 167 | [b]312 | 1,866 | 3.565 | 48.00 | 3.70 | 6.92 | 41.38 |
| Average | | | ...... | ...... | 4,509 | 2,170 | 161 | 313 | 1,865 | 3,573 | 48.13 | 3.57 | 6.94 | 41.36 |
| 207 | A | 3 | 2,974 | ...... | 4,509 | 1,841 | 204 | 368 | 2,096 | 3.457 | 40.82 | 4.52 | 8.17 | 46.49 |
| 207 | A | 4 | 4,892 | ...... | 4,521 | 1,888 | 199 | 357 | 2,077 | 3.482 | 41.77 | 4.40 | 7.90 | 45.93 |
| 207 | B | 3 | 2,798 | ...... | 4,509 | 1,875 | 224 | 378 | 2,032 | 3.378 | 41.58 | 4.97 | 8.38 | 45.07 |
| 207 | B | 4 | 4,630 | ...... | 4,521 | 1,918 | 208 | 355 | 2,040 | 3.456 | 42.42 | 4.61 | 7.84 | 45.13 |
| Average | | | ...... | ...... | 4,513 | 1,880 | 208 | 364 | 2,061 | 3.443 | 41.66 | 4.61 | 8.07 | 45.66 |
| Red-clover hay: | | | | | | | | | | | | | | |
| 179 | I | 1 | 4,459 | ...... | 4,450 | 1,940 | 306 | 309 | 1,895 | 3.373 | 43.61 | 6.87 | 6.94 | 42.58 |
| 179 | I | 2 | 3,144 | ...... | 4,426 | 1,857 | 288 | 324 | 1,957 | 3.452 | 41.95 | 6.50 | 7.33 | 44.22 |
| Average | | | ...... | ...... | 4,438 | 1,898 | 297 | 317 | 1,926 | 3,413 | 42.77 | 6.69 | 7.14 | 43.40 |
| 186 | I | 1a | 2,933 | ...... | 4,490 | 1,842 | 326 | 303 | 2,019 | 3,460 | 41.03 | 7.25 | 6.75 | 44.97 |
| 186 | I | 2a | 5,025 | ...... | 4,489 | 1,817 | 301 | 243 | 2,129 | 3,637 | 40.49 | 6.72 | 5.41 | 47.38 |
| 186 | I | 3a | 4,139 | ...... | 4,478 | 1,852 | 290 | 254 | 2,082 | 3.578 | 41.36 | 6.48 | 5.68 | 46.48 |
| Average | | | ...... | ...... | 4,486 | 1,837 | 306 | 267 | 2,076 | 3.558 | 40.95 | 6.82 | 5.95 | 46.28 |
| Mixed hay: | | | | | | | | | | | | | | |
| 211 | D | 1 | 6,204 | ...... | 4,400 | 2,077 | 209 | 285 | 1,829 | 3.442 | 47.20 | 4.75 | 6.49 | 41.56 |
| 211 | D | 4 | 3,498 | ...... | 4,391 | 1,837 | 234 | 332 | 1,990 | 3.406 | 41.83 | 5.33 | 7.54 | 45.30 |
| 211 | D | 5 | 1,786 | ...... | 4,390 | 1,956 | 255 | 345 | 1,834 | 3.294 | 44.57 | 5.80 | 7.85 | 41.78 |
| 211 | G | 1 | 6,092 | ...... | 4,393 | 1,906 | 208 | 300 | 1,979 | 3.463 | 43.39 | 4.75 | 6.82 | 45.04 |
| 211 | G | 4 | 3,149 | ...... | 4,391 | 1,878 | 220 | 319 | 1,974 | 3.420 | 42.76 | 5.00 | 7.27 | 44.97 |
| 211 | G | 5 | 1,608 | ...... | 4,390 | 1,917 | 238 | 358 | 1,877 | 3.313 | 43.68 | 5.41 | 8.15 | 42.76 |
| Average | | | ...... | ...... | 4,393 | 1,929 | 227 | 323 | 1,914 | 3.390 | 43.92 | 5.17 | 7.35 | 43.56 |
| Alfalfa hay: | | | | | | | | | | | | | | |
| 208 | D | 1 | 2,155 | ...... | 4,407 | 2,124 | 244 | 262 | 1,777 | 3.529 | 48.21 | 5.54 | 5.93 | 40.32 |
| 208 | D | 2 | 1,300 | ...... | 4,407 | 2,186 | 266 | 273 | 1,682 | 3.437 | 49.61 | 6.03 | 6.17 | 38.19 |
| 208 | E | 4 | 4,170 | ...... | 4,410 | 2,043 | 231 | 241 | 1,895 | 3.697 | 46.33 | 5.24 | 5.46 | 42.97 |
| 208 | E | 5 | 2,408 | ...... | 4,407 | 2,105 | 249 | 270 | 1,777 | 3.518 | 47.78 | 5.64 | 6.26 | 40.32 |
| 208 | E | 6 | 1,413 | ...... | 4,406 | 2,037 | 248 | 281 | 1,840 | 3.528 | 46.24 | 5.63 | 6.37 | 41.76 |
| 208 | C | 4 | 4,744 | ...... | 4,403 | 2,184 | 233 | 218 | 1,768 | 3.655 | 49.61 | 5.29 | 4.95 | 40.15 |
| 208 | C | 5 | 3,099 | ...... | 4,407 | 2,066 | 243 | 270 | 1,828 | 3.594 | 46.90 | 5.51 | 6.12 | 41.47 |
| 208 | C | 6 | 2,119 | ...... | 4,406 | 2,013 | 250 | 280 | 1,863 | 3.570 | 45.69 | 5.68 | 6.37 | 42.26 |
| Average | | | ...... | ...... | 4,407 | 2,095 | 246 | 262 | 1,804 | 3.567 | 47.54 | 5.58 | 5.94 | 40.94 |

[a] From analyses of individual samples for each period, and therefore differ somewhat from the averages of Table I.

[b] Corrected to N equilibrium, using Rubner's factor, 7.45 Calories per gram N.

[c] Computed by difference.

TABLE III.—*Losses of energy and their percentage distribution*—Continued

| Feeding stuff and experiment No. | Animal No. | Period No. | Dry matter eaten per day and head. | | Energy per kilogram of dry matter. | | | | | Metabolizable per kilogram of digestible organic matter. | Percentage losses. | | | Percentage metabolizable. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Coarse feed. | Concentrates. | Total. | Losses. In feces. | Losses. In urine. | Losses. In $CH_4$. | Metabolizable. | | In feces. | In urine. | In $CH_4$. | |
| | | | *Gm.* | *Gm.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | | | | |
| Alfalfa hay—Con. | | | | | | | | | | | | | | |
| 209 | F | 4 | 6,174 | ...... | 4,359 | 2,046 | 218 | 248 | 1,847 | 3,652 | 46.95 | 5.00 | 5.69 | 42.36 |
| 209 | F | 5 | 3,562 | ...... | 4,328 | 1,961 | 244 | 264 | 1,859 | 3,588 | 45.31 | 5.63 | 6.11 | 42.96 |
| 209 | F | 6 | 2,226 | ...... | 4,328 | 2,078 | 246 | 281 | 1,723 | 3,470 | 48.01 | 5.67 | 6.49 | 39.83 |
| Average | | | | ...... | 4,338 | 2,028 | 236 | 264 | 1,810 | 3,570 | 46.75 | 5.44 | 6.09 | 41.72 |
| 212 | H | 1 | 6,638 | ...... | 4,354 | 1,796 | 275 | 277 | 2,006 | 3,550 | 41.26 | 6.31 | 6.37 | 46.06 |
| 212 | H | 3 | 5,320 | ...... | 4,338 | 1,805 | 270 | 283 | 1,980 | 3,525 | 41.60 | 6.23 | 6.53 | 45.64 |
| 212 | H | 5 | 3,052 | ...... | 4,413 | 1,642 | 290 | 299 | 2,181 | 3,729 | 37.21 | 6.58 | 6.78 | 49.43 |
| Average | | | ...... | ...... | 4,368 | 1,748 | 278 | 286 | 2,056 | 3,601 | 40.02 | 6.36 | 6.55 | 47.07 |
| Alfalfa meal: | | | | | | | | | | | | | | |
| 212 | H | 2 | 6,671 | ...... | 4,364 | 1,889 | 252 | 250 | 1,973 | 3,671 | 43.29 | 5.78 | 5.74 | 45.19 |
| 212 | H | 4 | 5,408 | ...... | 4,379 | 1,897 | 250 | 259 | 1,973 | 3,646 | 43.31 | 5.72 | 5.93 | 45.04 |
| 212 | H | 6 | 3,155 | ...... | 4,379 | 1,726 | 270 | 292 | 2,091 | 3,727 | 39.41 | 6.18 | 6.66 | 47.75 |
| Average | | | ...... | ...... | 4,374 | 1,838 | 257 | 267 | 2,012 | 3,681 | 42.01 | 5.89 | 6.11 | 45.99 |
| Maize stover: | | | | | | | | | | | | | | |
| 210 | D | 1 | 4,335 | ...... | 4,333 | 1,860 | 172 | 335 | 1,966 | 3,488 | 42.93 | 3.96 | 7.74 | 45.37 |
| 210 | D | 2 | 3,548 | ...... | 4,327 | 1,837 | 185 | 335 | 1,970 | 3,493 | 42.47 | 4.27 | 7.75 | 45.51 |
| 210 | D | 3 | 2,563 | ...... | 4,337 | 1,867 | 195 | 354 | 1,921 | 3,369 | 43.06 | 4.50 | 8.15 | 44.29 |
| Average | | | ...... | ...... | 4,332 | 1,855 | 184 | 341 | 1,952 | 3,450 | 42.82 | 4.24 | 7.88 | 45.06 |
| Maize meal: | | | | | | | | | | | | | | |
| 179 | I | 3 | 3,163 | 735 | 4,360 | 633 | 441 | 488 | 2,798 | 3,186 | 14.52 | 10.12 | 11.20 | 64.16 |
| 179 | I | 4 | 3,186 | 3,451 | 4,366 | 401 | 167 | 406 | 3,392 | 3,716 | 9.18 | 3.83 | 9.31 | 77.68 |
| Average | | | ...... | ...... | 4,363 | 517 | 304 | 447 | 3,095 | 3,451 | 11.85 | 6.97 | 10.25 | 70.93 |
| 211 | G | 2 | 790 | 1,542 | 4,526 | 382 | 175 | 509 | 3,460 | 3,825 | 8.45 | 3.87 | 11.25 | 76.43 |
| 211 | G | 3 | 2,383 | 4,644 | 4,508 | 948 | 125 | 372 | 3,063 | 3,928 | 21.04 | 2.78 | 8.25 | 67.93 |
| Average | | | ...... | ...... | 4,517 | 665 | 150 | 441 | 3,261 | 3,877 | 14.74 | 3.32 | 9.75 | 72.19 |
| Wheat bran: | | | | | | | | | | | | | | |
| 190 | A | 1 | 1,978 | 1,370 | 4,545 | 1,484 | 230 | 365 | 2,466 | 4,003 | 32.67 | 5.06 | 8.04 | 54.24 |
| 190 | A | 2 | 1,996 | 2,583 | 4,558 | 1,440 | 221 | 311 | 2,586 | 3,982 | 31.59 | 4.85 | 6.82 | 56.74 |
| 190 | B | 1 | 1,712 | 999 | 4,563 | 1,500 | 254 | 332 | 2,477 | 3,951 | 32.87 | 5.56 | 7.28 | 54.29 |
| 190 | B | 2 | 1,779 | 1,803 | 4,461 | 1,336 | 271 | 340 | 2,514 | 3,872 | 29.96 | 6.07 | 7.62 | 56.35 |
| Average | | | ...... | ...... | 4,532 | 1,440 | 244 | 337 | 2,511 | 3,954 | 31.77 | 5.38 | 7.44 | 55.41 |
| Grain mixture No. 1: | | | | | | | | | | | | | | |
| 200 | A | 1 | 2,608 | 1,792 | 4,698 | 611 | 358 | [a] 390 | 3,339 | 3,946 | 13.00 | 7.62 | 8.30 | 71.08 |
| 200 | A | 2 | 2,631 | 4,173 | 4,695 | 1,051 | 288 | [a] 358 | 2,998 | 3,974 | 22.38 | 6.13 | 7.63 | 63.86 |
| 200 | B | 1 | 2,433 | 1,195 | 4,697 | 639 | 385 | [a] 381 | 3,294 | 3,923 | 13.60 | 8.19 | 8.11 | 70.10 |
| 200 | B | 2 | 2,454 | 2,129 | 4,695 | 1,064 | 322 | [a] 343 | 2,966 | 3,998 | 22.66 | 6.86 | 7.30 | 63.18 |
| Average | | | ...... | ...... | 4,696 | 841 | 338 | 368 | 3,149 | 3,960 | 17.91 | 7.20 | 7.84 | 67.05 |
| 207 | A | 1 | 2,935 | 1,996 | 4,658 | 951 | 316 | 397 | 2,994 | 3,876 | 20.42 | 6.78 | 8.52 | 64.28 |
| 207 | A | 2 | 2,949 | 4,759 | 4,688 | 1,019 | 294 | 342 | 3,033 | 3,991 | 21.73 | 6.28 | 7.29 | 64.70 |
| 207 | B | 1 | 2,761 | 1,398 | 4,658 | 1,104 | 363 | 439 | 2,752 | 3,683 | 23.71 | 7.79 | 9.42 | 59.08 |
| 207 | B | 2 | 2,774 | 2,677 | 4,688 | 791 | 353 | 409 | 3,135 | 3,888 | 16.87 | 7.53 | 8.72 | 66.88 |
| Average | | | ...... | ...... | 4,673 | 966 | 332 | 397 | 2,978 | 3,860 | 20.68 | 7.09 | 8.48 | 63.75 |

[a] Computed from digestible carbohydrates.

TABLE III.—*Losses of energy and their percentage distribution*—Continued

| Feeding stuff and experiment No. | Animal No. | Period No. | Dry matter eaten per day and head. Coarse feed. | Concentrates. | Energy per kilogram of dry matter. Total. | Losses. In feces. | In urine. | In $CH_4$. | Metabolizable. | Metabolizable per kilogram of digestible organic matter. | Percentage losses. In feces. | In urine. | In $CH_4$. | Percentage metabolizable. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Gm.* | *Gm.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | | | | |
| Grain mixture No. 2: | | | | | | | | | | | | | | |
| 208 | F | 1 | 1,086 | 2,122 | 4,607 | 1,258 | 173 | 349 | 2,827 | 3,887 | 27.32 | 3.75 | 7.58 | 61.36 |
| 208 | E | 2 | 590 | 1,163 | 4,611 | 840 | 202 | 442 | 3,127 | 3,837 | 18.22 | 4.38 | 9.57 | 67.83 |
| 208 | E | 3 | 387 | 764 | 4,595 | 901 | 236 | 346 | 3,112 | 3,890 | 19.61 | 5.14 | 7.54 | 67.72 |
| 208 | C | 2 | 731 | 1,453 | 4,611 | 1,246 | 192 | 463 | 2,710 | 3,706 | 27.03 | 4.16 | 10.04 | 58.77 |
| 208 | C | 3 | 521 | 1,018 | 4,594 | 826 | 242 | 486 | 3,038 | 3,711 | 17.98 | 5.28 | 10.58 | 66.16 |
| Average | | | | | 4,604 | 1,014 | 209 | 417 | 2,964 | 3,806 | 22.03 | 4.54 | 9.06 | 64.37 |
| 209 | F | 1 | 1,462 | 3,040 | 4,629 | 1,312 | 170 | 283 | 2,864 | 3,979 | 28.35 | 3.68 | 6.11 | 61.86 |
| 209 | F | 2 | 894 | 1,862 | 4,605 | 1,032 | 184 | 327 | 3,062 | 3,980 | 22.40 | 3.99 | 7.10 | 66.51 |
| 209 | F | 3 | 536 | 1,112 | 4,604 | 891 | 223 | 376 | 3,114 | 3,896 | 19.36 | 4.85 | 8.16 | 67.63 |
| Average | | | | | 4,613 | 1,078 | 193 | 329 | 3,013 | 3,952 | 23.37 | 4.17 | 7.12 | 65.34 |
| Hominy chop: | | | | | | | | | | | | | | |
| 211 | D | 2 | 1,747 | 1,764 | 4,720 | 542 | 195 | 496 | 3,487 | 3,993 | 11.48 | 4.13 | 10.50 | 73.89 |
| 211 | D | 3 | 3,910 | 3,949 | 4,698 | 603 | 167 | 371 | 3,557 | 4,156 | 12.83 | 3.56 | 7.90 | 75.71 |
| Average | | | | | 4,709 | 573 | 181 | 433 | 3,522 | 4,075 | 12.15 | 3.84 | 9.20 | 74.81 |
| Alfalfa hay and grain mixture No. 2: | | | | | | | | | | | | | | |
| 208 | E | 1 | 1,086 | 2,122 | 4,531 | 1,526 | 195 | 318 | 2,492 | 3,810 | 33.69 | 4.30 | 7.01 | 55.00 |
| 208 | E | 2 | 590 | 1,163 | 4,535 | 1,247 | 214 | 380 | 2,694 | 3,777 | 27.50 | 4.72 | 8.37 | 59.41 |
| 208 | E | 3 | 387 | 764 | 4,532 | 1,291 | 237 | 316 | 2,688 | 3,824 | 28.49 | 5.23 | 6.98 | 59.30 |
| 208 | C | 2 | 731 | 1,453 | 4,543 | 1,536 | 208 | 390 | 2,409 | 3,687 | 33.81 | 4.58 | 8.61 | 53.00 |
| 208 | C | 3 | 521 | 1,018 | 4,536 | 1,264 | 242 | 406 | 2,624 | 3,696 | 27.86 | 5.33 | 8.95 | 57.86 |
| Average | | | | | 4,535 | 1,373 | 219 | 362 | 2,581 | 3,759 | 30.27 | 4.83 | 7.98 | 56.92 |
| 209 | F | 1 | 1,462 | 3,040 | 4,527 | 1,540 | 189 | 275 | 2,523 | 3,872 | 34.67 | 4.26 | 6.18 | 54.89 |
| 209 | F | 2 | 894 | 1,862 | 4,516 | 1,352 | 199 | 305 | 2,660 | 3,888 | 29.94 | 4.40 | 6.74 | 58.92 |
| 209 | F | 3 | 536 | 1,112 | 4,515 | 1,259 | 225 | 338 | 2,693 | 3,826 | 27.88 | 4.99 | 7.48 | 59.65 |
| Average | | | | | 4,519 | 1,384 | 204 | 306 | 2,625 | 3,862 | 30.62 | 4.51 | 6.77 | 58.09 |
| Mixed hay and maize meal: | | | | | | | | | | | | | | |
| 211 | G | 2 | 790 | 1,542 | 4,483 | 897 | 189 | 443 | 2,954 | 3,701 | 20.00 | 4.22 | 9.88 | 65.90 |
| 211 | G | 3 | 2,383 | 4,644 | 4,468 | 1,271 | 156 | 352 | 2,689 | 3,763 | 28.45 | 3.49 | 7.88 | 60.18 |
| Average | | | | | 4,476 | 1,084 | 173 | 398 | 2,821 | 3,732 | 24.22 | 3.87 | 8.89 | 63.02 |
| Mixed hay and hominy chop: | | | | | | | | | | | | | | |
| 211 | D | 2 | 1,747 | 1,764 | 4,560 | 1,261 | 209 | 403 | 2,687 | 3,775 | 27.65 | 4.58 | 8.84 | 58.93 |
| 211 | D | 3 | 3,910 | 3,949 | 4,553 | 1,293 | 190 | 340 | 2,724 | 3,879 | 28.40 | 4.30 | 7.47 | 59.83 |
| Average | | | | | 4,557 | 1,277 | 203 | 371 | 2,706 | 3,827 | 28.02 | 4.44 | 8.15 | 59.39 |

TABLE IV.—*Average losses of chemical energy—metabolizable energy*

| Kind of feed and experiment No. | Gross energy per kilogram of dry matter. | Losses of chemical energy per kilogram of dry matter. | Metabolizable energy. | |
|---|---|---|---|---|
| | | | Per kilogram of dry matter. | Per kilogram of digestible organic matter. |
| Timothy hay: | *Calories.* | *Calories.* | *Calories.* | *Calories.* |
| 174 | 4,554 | 2,880 | 1,674 | 3,483 |
| 190 | 4,494 | 2,679 | 1,815 | 3,448 |
| 200 | 4,509 | 2,644 | 1,865 | 3,573 |
| 207 | 4,513 | 2,452 | 2,061 | 3,443 |
| Average | 4,518 | 2,664 | 1,854 | 3,487 |
| Red clover hay: | | | | |
| 179 | 4,438 | 2,512 | 1,926 | 3,413 |
| 186 | 4,486 | 2,410 | 2,076 | 3,558 |
| Average | 4,462 | 2,461 | 2,001 | 3,486 |
| Mixed hay: | | | | |
| 211 | 4,393 | 2,479 | 1,914 | 3,390 |
| Alfalfa hay: | | | | |
| 208 | 4,407 | 2,603 | 1,804 | 3,567 |
| 209 | 4,338 | 2,528 | 1,810 | 3,570 |
| 212 | 4,368 | 2,312 | 2,056 | 3,601 |
| Average | 4,371 | 2,481 | 1,890 | 3,579 |
| Alfalfa meal: | | | | |
| 212 | 4,374 | 2,362 | 2,012 | 3,681 |
| Average of alfalfa hay and meal | 4,372 | 2,451 | 1,921 | 3,605 |
| Maize stover: | | | | |
| 210 | 4,332 | 2,380 | 1,952 | 3,450 |
| Maize meal: | | | | |
| 179 [a] | 4,366 | 974 | 3,392 | 3,716 |
| 211 | 4,517 | 1,256 | 3,261 | 3,877 |
| Average | 4,442 | 1,115 | 3,327 | 3,797 |
| Wheat bran: | | | | |
| 190 | 4,532 | 2,021 | 2,511 | 3,954 |
| Grain mixture No. 1: | | | | |
| 200 | 4,696 | 1,547 | 3,149 | 3,960 |
| 207 | 4,673 | 1,695 | 2,978 | 3,860 |
| Average | 4,685 | 1,621 | 3,064 | 3,910 |
| Grain mixture No. 2: | | | | |
| 208 | 4,604 | 1,640 | 2,964 | 3,806 |
| 209 | 4,613 | 1,600 | 3,013 | 3,952 |
| Average | 4,609 | 1,620 | 2,989 | 3,879 |
| Hominy chop: | | | | |
| 211 | 4,709 | 1,187 | 3,522 | 4,075 |

[a] Period 4.

The results recorded in Table III illustrate the familiar fact that the greatest loss of chemical energy, especially in the case of coarse feeds, is that in the undigested feed residues of the feces and in the relatively small amounts of excretory products which, in the case of cattle, accompany them. The relative proportions lost in the urine and in the methane naturally vary with the composition of the feed, one rich in protein tending especially to increase the energy content of the urine, while carbohydrates tend to increase the excretion of methane.

Of greater interest, however, is the variability of the losses suffered by the same feeding stuff in different periods.

## INFLUENCE OF QUANTITY OF FEED CONSUMED ON LOSSES OF CHEMICAL ENERGY

In considering this question it should be borne in mind that the comparisons here reported are in every instance between *different amounts of the same ration*—i. e., they deal with the influence of quantity only and do not touch the question of the influence of heavy grain feeding. Furthermore, they relate to comparatively light feeding, many of the periods having been upon submaintenance rations, while the total dry matter of the feed seldom reached 18 pounds per 1,000 pounds of live weight. The experiments recorded in Table III include 31 cases in which different amounts either of a single feeding stuff or of an identical mixed ration were consumed by the same animal in two different periods of the same experiment, under conditions as nearly uniform as it was possible to make them. The results may be most conveniently compared on the basis of the percentage distribution of the energy, as shown in the last four columns of the table. In the following comparisons the results computed by difference for the concentrated feeds are not included.

### LOSSES IN METHANE

In a single instance (alfalfa hay and grain mixture No. 2 in experiment 208, steer E, periods 2 and 3) the percentage loss in the methane was greater on the heavier of the two rations and in another case (corn stover in experiment 210, steer D, periods 1 and 2) the difference was only 0.01 per cent. In two cases the determinations of methane are believed to have been inaccurate. In the remaining 29 cases the percentage loss in methane was distinctly greater on the lighter ration, the difference ranging from 0.11 to 2 per cent and tending, on the whole, to be somewhat greater on the mixed rations, with their larger proportion of readily soluble carbohydrates, than on those consisting exclusively of coarse fodder. In other words, as would be anticipated, the bacterial fermentation of the carbohydrates in the digestive tract of cattle proceeds to a distinctly greater extent on light than on heavy rations.

### LOSSES IN URINE

The percentage of the feed energy excreted in the urine was also greater on the lighter ration in 28 cases out of 33, the exceptions being two experiments on alfalfa hay (experiment 208, steer E, periods 5 and 6,

and experiment 212, steer H, periods 1 and 3), two on clover hay (experiment 179, steer I, periods 1 and 2, and experiment 186, steer I, periods 2a and 3a), and one on alfalfa meal (experiment 212, steer II, periods 2 and 4). In the remaining 28 cases there is one in which the difference amounts to only 0.02 per cent and two in which it is 0.04 per cent. In the remaining 25 it ranges from 0.12 to 0.78 per cent. This greater relative loss in the urine on the lighter ration can not be attributed to the presence of nitrogenous substances derived from an increased katabolism of body protein, since the energy of the urine has been at least approximately corrected to nitrogen equilibrium (p. 440). Since it is well established that the urine of cattle contains a considerable quantity of nonnitrogenous substances (2, p. 312–314, 320–322), it seems not impossible that the more extensive fermentation on the lighter ration may have resulted in an increase of these unknown constituents.

### LOSSES IN FECES

The results regarding the losses of chemical energy in the feces are by no means so uniform as in the case of the methane and of the urine. In 22 out of 33 cases there is a distinctly smaller relative loss of energy in the feces with the lighter ration—i. e., a greater apparent digestibility—the difference in the percentages ranging from 0.28 to 8.45. In the other third of the cases, however, the difference is in the opposite direction, ranging from 0.37 to 2.74, with the exception of one case of practical equality, so that it appears that other factors besides the extent of the methane fermentation affected the percentage digestibility. Two rather marked cases of a greater loss of energy in the feces on the lighter ration are found in experiment 211, periods 4 and 5, with relatively very small rations. Whether the relative loss in the feces increases or decreases with an increase of the ration seems to bear no relation to the total quantity of feed consumed either per head or per 500 kg. of live weight. The 10 instances in which a greater percentage loss in the feces was observed on the lighter of the two rations include, it is true, the more extreme rations as regards the total quantity, but the averages for the two groups are not widely different (4,376 and 3,952 gm. of dry matter).

### PERCENTAGE OF FEED ENERGY METABOLIZABLE

The bearing of the foregoing facts upon the percentage of the feed energy which is metabolizable is obvious. Clearly the fermentation which plays so large a rôle in the digestive processes of ruminants was relatively more intense on the lighter rations, resulting in the breaking down of a larger proportion of the carbohydrates and in a greater loss of chemical energy in the methane, accompanied in most instances by an increased loss in the urine also. On the other hand, however, the organic acids resulting from the fermentation are resorbed and oxidized in the body, and their energy, together with the heat evolved in the fermentation, constitutes part of the metabolizable energy of the feed as defined on page 439. Whether the proportion of the total energy of the feed which is metabolizable be greater or less on the lighter ration will depend, therefore, upon the nature of the additional carbohydrates fermented. If the increased fermentation attacks the more insoluble carbohydrates, which would otherwise escape digestion entirely and

reappear in the feces, the result will be a relative increase in the metabolizable energy. On the other hand, if the greater fermentation on the lighter rations is at the expense of the more soluble carbohydrates which would otherwise be digested by the enzyms of the intestines, the metabolizable energy will be diminished by the quantity of chemical energy escaping in the methane (and urine). It is perhaps not surprising, therefore, to find that the effect of the quantity of feed upon the percentage of energy metabolizable was somewhat variable.

In 19 out of 22 cases mentioned in the previous paragraph in which the percentage loss of energy in the feces decreased as the amount of feed consumed was diminished, the percentage of energy metabolized did in fact increase, while in the remaining 3 cases the greater losses in methane and urine overbalanced the effect of the increased digestibility. In each of the 10 instances in which the percentage loss of energy in the feces was greater on the lighter rations, the percentage metabolizable shows a corresponding decrease, so that of the entire 33 comparisons 14 show a greater and 19 a less percentage metabolizable on lighter as compared with heavier rations.

On the whole, then, the differences in amount of feed consumed, within the limits of these experiments, failed to show any unmistakable effect upon the quantity of energy actually liberated in the body from a unit weight of feed. Morever, it must be borne in mind that a considerable part of the additional energy secured by the more extensive fermentation of the lighter ration is liberated in the digestive tract as heat of fermentation and does not enter into the energy exchange of the body tissues, so that the difference in the net nutritive effect is likely to be less than that in the metabolizable energy as ordinarily defined.

## INFLUENCE OF INDIVIDUALITY ON LOSSES OF CHEMICAL ENERGY

In five of the experiments the same feeding stuff or mixture of feeding stuffs was fed to more than one animal, although unfortunately the amounts consumed were not the same either per head or in proportion to the live weight, so that exact comparisons are not possible.

In experiments 190, 200, and 207 a pure-bred Shorthorn steer was compared with a so-called scrub. A comparison of the averages for the lighter and the heavier rations of timothy hay, respectively, for the three successive years gives the averages shown in Table V, which fail to show any distinct individual difference between the two animals. The results computed by difference for the wheat bran in experiment 190 and for the grain mixture No. 1 in experiments 200 and 207 show somewhat larger numerical differences, but when the errors incident to such calculations by difference are considered they agree with those upon hay in showing no material difference between these two animals. This point has been discussed from a slightly different standpoint elsewhere (10).

TABLE V.—*Influence of individuality of cattle on losses of chemical energy*

| Character of experiment. | Loss of energy. In feces. | Loss of energy. In urine. | Loss of energy. In methane. | Metabolizable. |
|---|---|---|---|---|
| Timothy hay (experiments 190, 200, and 207): | | | | |
| Average of light rations— | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| Steer A | 46.03 | 3.87 | 7.34 | 42.76 |
| Steer B | 46.03 | 3.96 | 7.79 | 42.22 |
| Difference | 0 | -.09 | -.45 | +.54 |
| Average of heavy rations— | | | | |
| Steer A | 47.69 | 3.55 | 7.11 | 41.65 |
| Steer B | 46.32 | 3.70 | 7.18 | 42.80 |
| Difference | +1.37 | -.15 | -.07 | -1.15 |
| Average of all rations— | | | | |
| Steer A | 46.86 | 3.71 | 7.22 | 42.21 |
| Steer B | 46.18 | 3.83 | 7.49 | 42.50 |
| Difference | +.68 | -.12 | -.27 | -.29 |
| Alfalfa hay (experiment 208): | | | | |
| Average of medium and light rations— | | | | |
| Steer D (periods 1 and 2) | 48.91 | 5.79 | 6.05 | 39.25 |
| Steer E (periods 5 and 6) | 47.01 | 5.64 | 6.31 | 41.04 |
| Steer C (periods 5 and 6) | 46.30 | 5.60 | 6.24 | 41.86 |
| Difference (D−C) | +2.61 | +.19 | -.19 | -2.61 |
| Heavy rations— | | | | |
| Steer E (period 4) | 46.33 | 5.24 | 5.46 | 42.97 |
| Steer C (period 4) | 49.61 | 5.29 | 4.95 | 40.15 |
| Difference | -3.28 | -.05 | +.51 | +2.82 |
| Alfalfa hay and grain (experiment 208): | | | | |
| Average of medium and light rations— | | | | |
| Steer E (periods 2 and 3) | 28.00 | 4.98 | 7.67 | 59.35 |
| Steer C (periods 2 and 3) | 30.83 | 4.96 | 8.78 | 55.43 |
| Difference | -2.83 | +.02 | -1.11 | +3.92 |
| Mixed hay (experiment 211): | | | | |
| Steer D (periods 1, 4, and 5) | 44.53 | 5.29 | 7.30 | 42.88 |
| Steer G (periods 1, 4, and 5) | 43.28 | 5.05 | 7.41 | 44.26 |
| Difference | +1.25 | +.24 | -.11 | -1.38 |

The three animals used in experiment 208 had been subjected to different previous treatment, steer D having received almost from birth a restricted quantity of feed, steer E a ration ample to support normal growth, and steer C as heavy feeding as practicable. In the periods on the medium and light rations of alfalfa hay these animals showed slight differences in their digestive powers in the order named, the average

results for the two quantities being as shown in Table V. On the other hand, upon the heavy hay ration and also upon the mixed ration of hay and grain steer C was distinctly inferior to steer E, losing more chemical energy in both methane and feces. This was the most distinct individual difference in these experiments. It should perhaps be noted that steer C showed a tendency to bloat and to get off feed on heavy rations and possibly did not have full normal digestive power. In experiment 211 steer D, then a year older, on the average of three periods on mixed hay again showed a slight inferiority to another animal which presumably had received better feeding during growth.

Clearly individual differences between the animals had no very material influence on the losses of chemical energy in these experiments. In most instances the differences are well within the limits of error for such determinations, and even in those cases where there seem to be distinct individual differences they are comparatively slight, being of about the same magnitude as those observed by G. Kühn (28) and rather smaller than those found by Armsby (1) in experiments on three steers.

VARIABILITY OF METABOLIZABLE ENERGY

The results recorded in Tables III and IV show clearly that the metabolizable energy of a feeding stuff is by no means a constant. Not only do the averages for feeding stuffs of the same name differ more or less, but the metabolizable energy of the same sample is more or less variable in the different periods.

The losses of chemical energy which a feeding stuff suffers are substantially determined by the nature and extent of the digestive processes. Digestibility, however, especially in ruminants, is a very complex affair, depending on many factors. Broadly speaking, it may be characterized as a series of fermentations, effected in part by a variety of organized ferments and in part by enzyms secreted by the digestive organs or contained in the feed itself. Changes in the composition of the contents of the digestive tract or in the rapidity with which they move forward through it can hardly fail to influence in a variety of ways the course of these fermentations, and it seems on the whole rather surprising that they go forward as uniformly as they do.

In these experiments they appear to have been affected chiefly by the variations in the amount of feed consumed. Recently Zuntz and his associates (20, 53) have reported striking instances in which the extent of the methane fermentation in particular has been markedly affected by the make-up of the rations and especially by the order in which the feeds were consumed, while Völtz and his associates (47, 48) have laid much stress on the practical importance of these results. No such marked differences occurred in our experiments, but, on the other hand, the range of feeds was not so wide. It is perhaps too early

to judge the full significance of Zuntz's results, but they should at least serve to correct the notion, more or less subconsciously held by not a few, of digestion as a perfectly definite process and of a digestion coefficient as a sort of chemical constant. On the other hand, however, it is easy to overestimate the importance of these variations in the digestive process in their bearing upon estimates of the values of feeding stuffs. On the whole, they appear to be of far less significance than other factors to be considered later.

## ESTIMATION OF METABOLIZABLE ENERGY

### FROM METABOLISM EXPERIMENTS

The losses of chemical energy in feces and urine are readily determined by means of the ordinary metabolism experiment, but the determination of the losses in the combustible gases requires special and somewhat costly apparatus. A number of experimenters have therefore attempted to estimate the amounts of these gases produced, usually from the amounts of carbohydrates (crude fiber and nitrogen-free extract) digested, using, as a rule, the average factor derived from Kellner's investigations (26, p. 420)—viz, 4.2 parts of $CH_4$ per 100 parts of digested carbohydrates.

Our experiments have yielded somewhat higher figures, as shown in Table VI, giving the maximum, minimum, and average results for each feeding stuff or mixture.

TABLE VI.—*Quantity of methane per 100 gm. of digestible carbohydrates*

| Feeding stuff. | Number of experiments. | Quantity of methane. | | |
|---|---|---|---|---|
| | | Maximum. | Minimum. | Average. |
| | | *Gm.* | *Gm.* | *Gm.* |
| Timothy hay | 12 | 3.8 | 5.1 | 4.6 |
| Clover hay | 5 | 3.9 | 5.2 | 4.6 |
| Mixed hay | 6 | 4.6 | 5.8 | 5.1 |
| Alfalfa hay | 17 | 4.2 | 5.3 | 4.8 |
| Maize stover | 3 | 4.7 | 4.8 | 4.7 |
| Average | 43 | | | 4.8 |
| Maize meal and clover hay | 2 | 4.3 | 5.2 | 4.8 |
| Wheat bran and timothy hay | 4 | 4.8 | 5.2 | 4.9 |
| Grain mixture No. 1 and timothy hay | 4 | 4.7 | 5.3 | 5.0 |
| Grain mixture No. 2 and alfalfa hay | 8 | 3.8 | 5.5 | 4.5 |
| Maize meal and mixed hay | 2 | 4.2 | 4.7 | 4.5 |
| Hominy chop and mixed hay | 2 | 4.4 | 5.0 | 4.7 |
| Average | 22 | | | 4.7 |
| Average of author's experiments | 65 | 3.8 | 5.5 | 4.8 |
| Average of Kellner's experiments | 44 | 2.9 | 5.5 | 4.2 |

While there is a considerable range in the results of individual experiments, nevertheless an estimate of 4.5 grams of $CH_4$ per 100 gm. of digested carbohydrates affords a fair basis for an approximate estimate of the losses of chemical energy in the combustible gases and for computing the metabolizable energy of a feeding stuff by means of the ordinary digestion experiment combined with the collection of the urine and a determination of the heats of combustion of the visible excreta. The additional labor required for this purpose is so small that it is to be hoped that in future digestion experiments it may be undertaken whenever possible.

### FROM DIGESTIBLE ORGANIC MATTER

It is possible also to estimate the average metabolizable energy of a feeding stuff from its content of total digestible organic matter, as shown by the ordinary feeding tables.

The differences shown in Tables III and IV between the metabolizable energy per kilogram of dry matter of the different feeding stuffs are due chiefly, as already pointed out, to differences in the proportion of the chemical energy carried off in the feces, so that the metabolizable energy per kilogram of digestible organic matter shows a striking degree of uniformity as between different coarse feeds, on one hand, and as between different concentrates, on the other, a fact quite in harmony with earlier results reported by Kellner (26). Expressing the results in therms per kilogram and using for the apparent metabolizable energy of Kellner and Köhler's feeding stuffs the figures computed by Armsby (2, p. 301), we obtain the following averages:

*Metabolizable energy per kilogram of digestible organic matter*

COARSE FEEDS

| | Therms. |
|---|---|
| Armsby and Fries: | |
| Timothy hay | 3.49 |
| Red clover hay | 3.49 |
| Mixed hay | 3.39 |
| Alfalfa hay and meal | 3.61 |
| Maize stover | 3.45 |
| Average | 3.48 |
| Kellner and Köhler: | |
| Meadow hay | 3.50 |
| Oat straw | 3.74 |
| Wheat straw | 3.31 |
| Extracted straw | 3.64 |
| Average | 3.55 |

CONCENTRATES

| | Therms. |
|---|---|
| Armsby and Fries: | |
| Maize meal | 3.80 |
| Wheat bran | 3.99 |
| Grain mixture No. 2 | 3.88 |
| Average | 3.89 |
| Grain mixture No. 1 | 3.91 |
| Hominy chop | 4.08 |
| Average | 4.00 |
| Kellner and Köhler: | |
| Beet molasses | 3.47 |
| Starch | 3.60 |
| Wheat gluten | 4.79 |

Tangl (44), Tangl and Weiser (45), and Zaitschek (50) have also determined the metabolizable energy of a number of feeding stuffs for cattle, the methane being estimated from the amount of digestible carbohydrates, with the following results, which are very similar to those just reported:

*Metabolizable energy per kilogram of digestible organic matter: Tangl's experiments*

| | Therms. |
|---|---|
| Meadow hay | 3.44 |
| Ensiled meadow hay | 3.70 |
| Hay from irrigated meadows | 3.60 |
| Broom-corn millet meal | 3.68 |
| Pumpkins | 4.29 |

On the other hand, four experiments upon a bull by Völtz et al. (48), in which the production of methane was likewise computed, gave notably higher figures, viz:

*Metabolizable energy per kilogram of digestible organic matter: Völtz's experiments*

| | Therms. |
|---|---|
| Mixed ration (hay, straw, malt sprouts, dried brewers' grains, and potato flakes) | 3.95 |
| Dried distillery residue (from potatoes) | 4.84 |
| Palm-nut meal | 4.85 |
| Beet molasses | 4.36 |

The most important factor influencing the metabolizable energy of the digestible organic matter of concentrates seems to be the percentage of fat in the feeding stuff, as appears from a comparison of the data contained in Table I, while feeding stuffs exceptionally high in protein have also a high content of metabolizable energy in their digestible matter, as in the case of Kellner and Köhler's wheat gluten. There seems no obvious explanation of the exceptionally high results obtained by Völtz, but it would seem that for the present, with the ordinary dry feeding stuffs or mixtures, the following factors may safely be made the basis for computing approximately the metabolizable energy of feeding stuffs for cattle when their content of digestible organic matter is known or can be estimated.

*Metabolizable energy of feeding stuffs per kilogram of digestible organic matter*

| | Therms. |
|---|---|
| Coarse feeds | 3.5 |
| Concentrated feeds: | |
| With less than 5 per cent of digestible fat | 3.9 |
| With more than 5 per cent of digestible fat | 4.0 |

No similar results have been reported on succulent feeds, with the exception of Zaitschek's figure for pumpkins.

## II. EXPENDITURE OF ENERGY CONSEQUENT UPON FEED CONSUMPTION AND ITS FACTORS

That the consumption of feed tends to increase the metabolism of an animal has become a commonplace of physiology. The magnitude of the effect varies within rather wide limits according to the chemical and physical properties of the feed, while there is still more or less difference of opinion as to its causes. Zuntz and his associates have called it "work of digestion" and have attributed it largely to increased muscular and glandular activity of the digestive and excretory organs. Most physiological investigations in this field have been made on carnivora or on man, in which the increase of the metabolism is not usually very large, except when much protein is consumed. More recent investigations on these species appear to have shown that the mechanical work of the digestive organs is but a small factor and that the term "work of digestion" is not a fortunate one. With herbivora and especially with ruminants, however, the total effect on the metabolism is quantitatively very marked, while the mechanical factor is of much greater significance. This was early shown by Zuntz and Hagemann (52) in their investigations upon the horse. With ruminants the most extensive investigations are those made at the Möckern (Germany) Experiment Station by G. Kühn (29) and by Kellner (24, 25, 26, 27). These investigations, especially those of Kellner, were directed primarily to the determination of the relative values of nutrients and feeding stuffs, but from another point of view they constituted also determinations of the energy expenditure caused by the feed. The main purpose of the experiments here reported was to determine the proportion of the feed energy expended in the increased metabolism by means of direct measurements of the heat evolved, checked by determinations of the respiratory products, the relative values of the feeding stuffs being obtained by difference.

### DIFFERENCES IN MUSCULAR ACTIVITY

#### INFLUENCE OF STANDING OR LYING UPON METABOLISM

We have repeatedly called attention to the very striking increase in the heat elimination of cattle when standing as compared with that when lying and have shown (11), in reply to the criticism of Zuntz, that it is

accompanied by a substantially corresponding increase in the gaseous excretion.

Since conclusions regarding the influence of feed consumption on the heat production must be drawn from comparisons of two or more periods on different amounts of feed, it is obvious that the periods must be made as nearly identical in other respects as practicable. It being impossible to control the standing and lying of the subject, it became necessary, therefore, to attempt a quantitative determination of the influence of standing upon the metabolism of the animal as measured by its heat production and on this basis to correct the results of each period to some uniform ratio of standing to lying. It was natural to suppose that the increment of the metabolism during standing was due to the work of supporting the body in an upright position, but it soon became evident that it was also to a very considerable extent a function of the feed consumption.

The apparatus used in these experiments is a flow calorimeter, the temperature difference between the ingoing and the outcoming water being read every four minutes. Since the hydrothermal equivalent of the absorber system and the contained water is only 6 kg., it is possible to follow very closely the rate of elimination of heat by radiation and conduction. The results found by the authors (11) show that the heat carried off as latent heat of water vapor is substantially proportional to that eliminated by radiation and conduction. On this basis the average heat elimination per minute and for 24 hours during standing and lying has been computed for each 48-hour period (or, in experiments 174, 190, 200, and 207, from selected uniform intervals). The addition of the necessary correction for the gain or loss of matter by the body gives the average heat production when standing or lying, respectively, while the difference between the two represents the increment due to standing for 24 hours. The following example, taken from experiment 179, period 1, in which 76.65 per cent of the total heat emitted was given off by radiation and conduction and 23.35 per cent as latent heat of water vapor, and in which the correction for loss of matter by the body was −127 Calories, may serve to illustrate the method.

*Method of computing heat production of cattle when standing or lying*

| | | Standing. | Lying. |
|---|---|---|---|
| Time standing or lying | minutes.. | 1, 767 | 1, 113 |
| Heat emitted by radiation and conduction: | | | |
| Total | Calories.. | 12, 652 | 5. 128 |
| Per minute | Calories.. | 7. 160 | 4. 607 |
| Total heat production computed per 24 hours: | | | |
| Standing | $7.160 \times 1,440 \div 0.7665 - 127 =$ | 13, 324 | Calories. |
| Lying | $4.607 \times 1,440 \div 0.7665 - 127 =$ | 8, 528 | Calories. |
| Difference | | 4, 796 | Calories. |

The differences thus found between the heat production of the animals per 24 hours when standing and when lying were as shown in Table VII, in which are included for comparison the total dry matter consumed and the average live weight of the animal. In order to facilitate a general comparison of the results of the several experiments, the influence of the differences in live weight has also been eliminated to a degree by computing the data of Table V to a uniform body weight of 500 kg., with the results shown graphically in figure 1.

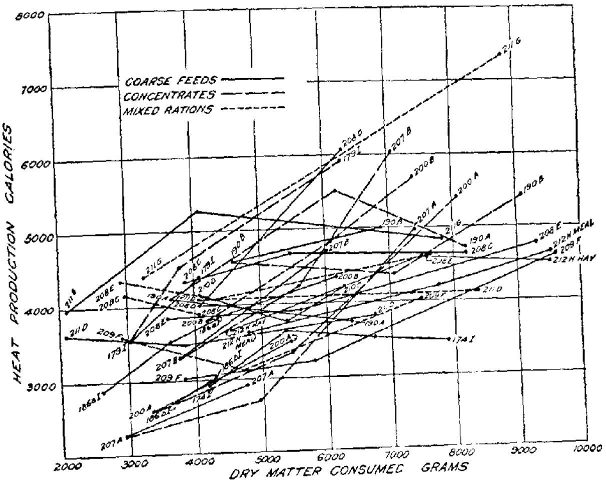


FIG. 1.—Graph showing the dry matter eaten and the increments of heat production due to standing; computed per 500 kg. live weight per 24 hours.

It should be clearly understood that both Table VII and figure 1 show the relation of feed consumption to the *heat increment during standing*, computed in the manner just illustrated, and not to the total heat production.

TABLE VII.—*Increments of heat production of cattle in standing*

| Feeding stuff and experiment. | Average live weight. | Dry matter eaten per head. | Increment of heat production per 24 hours. Per head. | Increment of heat production per 24 hours. Per kilogram of increment of dry matter eaten. |
|---|---|---|---|---|
| TIMOTHY HAY: | | | | |
| Experiment 174— | *Kilograms.* | *Grams.* | *Calories.* | *Calories.* |
| Steer I (period A) | 387 | 3, 237 | 2, 258 | |
| Steer I (period B) | 403 | 4, 373 | 2, 935 | 598 |
| Steer I (period C) | 416 | 5, 480 | 2, 913 | −20 |
| Steer I (period D) | 424 | 6, 440 | 2, 928 | 16 |
| Steer I (periods D–A) | | | | 210 |
| Experiment 190— | | | | |
| Steer A (period 3) | 269 | 2, 001 | 2, 206 | |
| Steer A (period 4) | 278 | 3, 493 | 2, 076 | −88 |
| Steer B (period 3) | 194 | 1, 774 | 1, 774 | |
| Steer B (period 4) | 190 | 2, 610 | 1, 899 | 148 |
| Experiment 200— | | | | |
| Steer A (period 3) | 399 | 2, 647 | 2, 052 | |
| Steer A (period 4) | 407 | 4, 425 | 2, 838 | 442 |
| Steer B (period 3) | 296 | 2, 470 | 2, 233 | |
| Steer B (period 4) | 309 | 3, 805 | 2, 695 | 346 |
| Experiment 207— | | | | |
| Steer A (period 3) | 507 | 2, 974 | 2, 293 | |
| Steer A (period 4) | 514 | 4, 892 | 2, 994 | 364 |
| Steer B (period 3) | 374 | 2, 798 | 2, 467 | |
| Steer B (period 4) | 385 | 4, 630 | 3, 618 | 628 |
| RED CLOVER HAY: | | | | |
| Experiment 179— | | | | |
| Steer 1 (period 1) | 545 | 4, 459 | 4, 794 | |
| Steer 1 (period 2) | 520 | 3, 144 | 3, 679 | 848 |
| Experiment 186 (series *a*)— | | | | |
| Steer 1 (period 1) | 571 | 2, 933 | 3, 247 | |
| Steer 1 (period 3) | 580 | 4, 139 | 4, 018 | 637 |
| Steer 1 (period 2) | 587 | 5, 025 | 4, 438 | 474 |
| Steer 1 (periods 2–1) | | | | 570 |
| Experiment 186 (series *b*)— | | | | |
| Steer 1 (period 3) | 566 | 4, 139 | 3, 080 | |
| Steer 1 (period 2) | 576 | 5, 025 | 3, 452 | 420 |
| MIXED HAY: | | | | |
| Experiment 211— | | | | |
| Steer D (period 1) | 460 | 6, 204 | 3, 515 | |
| Steer D (period 4) | 455 | 3, 498 | 3, 147 | 136 |
| Steer D (period 5) | 428 | 1, 786 | 3, 107 | 24 |
| Steer D (periods 1–5) | | | | 92 |
| Steer G (period 1) | 389 | 6, 092 | 3, 764 | |
| Steer G (period 4) | 387 | 3, 149 | 4, 066 | −102 |
| Steer G (period 5) | 364 | 1, 608 | 2, 887 | 764 |
| Steer G (periods 1–5) | | | | 196 |
| ALFALFA HAY: | | | | |
| Experiment 208— | | | | |
| Steer D (period 1) | 171 | 2, 155 | 2, 077 | |
| Steer D (period 2) | 162 | 1, 300 | 1, 314 | 894 |
| Steer E (period 4) | 224 | 4, 170 | 2, 145 | |
| Steer E (period 5) | 215 | 2, 408 | 1, 683 | 264 |
| Steer E (period 6) | 197 | 1, 413 | 1, 512 | 170 |
| Steer E (periods 4–6) | | | | 230 |
| Steer C (period 4) | 289 | 4, 744 | 2, 690 | |
| Steer C (period 5) | 284 | 3, 099 | 2, 659 | 18 |
| Steer C (period 6) | 275 | 2, 119 | 2, 380 | 286 |
| Steer C (periods 4–6) | | | | 118 |

TABLE VII.—*Increments of heat production of cattle in standing*—Continued

| Feeding stuff and experiment. | Average live weight. | Dry matter eaten per head. | Increment of heat production per 24 hours. | |
|---|---|---|---|---|
| | | | Per head. | Per kilogram of increment of dry matter eaten. |
| | *Kilograms.* | *Grams.* | *Calories.* | *Calories.* |
| ALFALFA HAY—Continued. | | | | |
| Experiment 209— | | | | |
| Steer F (period 4) | 321 | 6,174 | 2,973 | 372 |
| Steer F (period 5) | 308 | 3,562 | 1,997 | 172 |
| Steer F (period 6) | 292 | 2,226 | 1,768 | |
| Steer F (periods 4–6) | | | | 304 |
| Experiment 212— | | | | |
| Steer H (period 1) | 349 | 6,638 | 3,167 | −44 |
| Steer H (period 3) | 354 | 5,320 | 3,224 | 328 |
| Steer H (period 5) | 337 | 3,052 | 2,481 | |
| Steer H (periods 1–5) | | | | 192 |
| ALFALFA MEAL: | | | | |
| Experiment 212— | | | | |
| Steer H (period 2) | 349 | 6,671 | 3,246 | 242 |
| Steer H (period 4) | 349 | 5,408 | 2,942 | 252 |
| Steer H (period 6) | 329 | 3,155 | 2,377 | |
| Steer H (periods 2–6) | | | | 248 |
| MAIZE STOVER: | | | | |
| Experiment 210— | | | | |
| Steer D (period 1) | 345 | 4,335 | 2,919 | 550 |
| Steer D (period 2) | 331 | 3,548 | 2,486 | −134 |
| Steer D (period 3) | 316 | 2,563 | 2,617 | |
| Steer D (periods 1–3) | | | | 170 |
| MAIZE MEAL ADDED TO CLOVER HAY: | | | | |
| Experiment 179— | | | | |
| Steer I (period 2) | 520 | [a] 3,144 | 3,679 | 1,300 |
| Steer I (period 3) | 514 | 3,898 | 4,659 | 598 |
| Steer I (period 4) | 532 | 6,637 | 6,296 | |
| Steer I (periods 4–2) | | | | 748 |
| WHEAT BRAN ADDED TO TIMOTHY HAY: | | | | |
| Experiment 190— | | | | |
| Steer A (period 3) | 269 | [a] 2,001 | 2,206 | 586 |
| Steer A (period 1) | 271 | 3,348 | 2,995 | −288 |
| Steer A (period 2) | 279 | 4,579 | 2,639 | |
| Steer A (periods 2–3) | | | | 168 |
| Steer B (period 3) | 194 | [a] 1,774 | 1,774 | −32 |
| Steer B (period 1) | 199 | 2,803 | 1,741 | 540 |
| Steer B (period 2) | 197 | 3,582 | 2,135 | |
| Steer B (periods 2–3) | | | | 200 |
| GRAIN MIXTURE NO. 1 ADDED TO TIMOTHY HAY: | | | | |
| Experiment 200— | | | | |
| Steer A (periods 3) | 399 | [a] 2,647 | 2,052 | 374 |
| Steer A (period 1) | 404 | 4,400 | 2,708 | 765 |
| Steer A (period 2) | 421 | 6,804 | 4,547 | |
| Steer A (periods 2–3) | | | | 600 |
| Steer B (period 3) | 296 | [a] 2,470 | 2,233 | 565 |
| Steer B (period 1) | 298 | 3,628 | 2,887 | 736 |
| Steer B (period 2) | 310 | 4,583 | 3,591 | |
| Steer B (periods 2–3) | | | | 642 |

[a] Basal ration of coarse fodder only.

TABLE VII.—*Increments of heat production of cattle in standing*—Continued

| Feeding stuff and experiment. | Average live weight. | Dry matter eaten per head. | Increment of heat production per 24 hours. Per head. | Increment of heat production per 24 hours. Per kilogram of increment of dry matter eaten. |
|---|---|---|---|---|
| GRAIN MIXTURE NO. 1 ADDED TO TIMOTHY HAY—Continued. | | | | |
| Experiment 207— | *Kilograms.* | *Grams.* | *Calories.* | *Calories.* |
| Steer A (period 3) | 507 | [a] 2, 974 | 2, 293 | |
| Steer A (period 1) | 499 | 4, 931 | 2, 704 | 210 |
| Steer A (period 2) | 519 | 7, 708 | 5, 182 | 892 |
| Steer A (periods 2–3) | ......... | ......... | ......... | 610 |
| Steer B (period 3) | 374 | [a] 2, 798 | 2, 467 | |
| Steer B (period 1) | 373 | 4, 159 | 3, 165 | 513 |
| Steer B (period 2) | 386 | 5, 451 | 4, 659 | 1, 158 |
| Steer B (periods 2–3) | ......... | ......... | ......... | 828 |
| ALFALFA HAY AND GRAIN MIXTURE NO. 2: | | | | |
| Experiment 208— | | | | |
| Steer E (period 1) | 210 | 3, 208 | 1, 944 | |
| Steer E (period 2) | 206 | 1, 753 | 1, 682 | 180 |
| Steer E (period 3) | 197 | 1, 151 | 1, 718 | −60 |
| Steer E (periods 1–3) | ......... | ......... | ......... | 110 |
| Steer C (period 2) | 269 | 2, 184 | 2, 083 | |
| Steer C (period 3) | 259 | 1, 539 | 2, 154 | −110 |
| Experiment 209— | | | | |
| Steer F (period 1) | 301 | 4, 502 | 2, 442 | |
| Steer F (period 2) | 293 | 2, 756 | 1, 834 | 348 |
| Steer F (period 3) | 283 | 1, 648 | 2, 029 | −88 |
| Steer F (periods 1–3) | ......... | ......... | ......... | 144 |
| MIXED HAY AND MAIZE MEAL: | | | | |
| Experiment 211— | | | | |
| Steer G (period 2) | 358 | 2, 332 | 3, 158 | |
| Steer G (period 3) | 398 | 7, 027 | 5, 840 | 572 |
| MIXED HAY AND HOMINY CHOP: | | | | |
| Experiment 211— | | | | |
| Steer D (period 2) | 432 | 3, 511 | 3, 533 | |
| Steer D (period 3) | 470 | 7, 859 | 3, 905 | 86 |

[a] Basal ration of coarse fodder only.

A simple inspection of Table VII suffices to show that the increment of heat production in standing can not be due to any large extent to the muscular work of supporting the body, since, in the light of Zuntz and Hagemann's (52) experiments on the horse, it must be assumed that this would be at least approximately proportional to the weight of the animal, while, in fact, in a large majority of cases the difference between the periods is very much greater than the corresponding difference in live weight. Even on the extreme assumption that in the periods on minimum rations the heat increment in standing was due exclusively to the increased muscular effort, the differences in live weight do not even remotely account for the greater increments in the other periods.

RELATION TO AMOUNT OF FEED

In spite of the considerable variations in the individual results and of some negative values, it appears clear, both from Table VII and from figure 1, especially if the comparisons be made between the smallest and the greatest rations, that the effect of the feed in increasing the metabolism tended to be distinctly greater during standing than during lying, and that, on the whole, the differences tended to be greater with concentrates or with mixed rations than with coarse feeds. In other words, the difference between the metabolism of the animal when standing and when lying was relatively greater on the heavier than on the lighter rations.

This difference can hardly be ascribed to a greater direct stimulus of cell metabolism by the products of digestion. One explanation for it might be sought in the fact that the feed was consumed by the animal while standing. Experiments by Paechtner (39) and by Dahm (17) on cattle and by Ustjanzew (46) on sheep, in which the respiratory exchange was determined in short periods, showed that the mastication of 1 kg. of hay increased the metabolism of the animal by approximately 60 Calories. In our experiments this would be equivalent to the production during perhaps half an hour after feeding—i. e., at 6 a. m. and 6 p. m.—of from 12 to as much as 200 Calories of heat, or twice this amount in 24 hours, which amounts would be added to the standing metabolism. On the other hand, however, according to the same experimenters, the rumination of the feed would increase the heat production by, roughly, two-thirds as much, and this would constitute to a considerable extent an addition to the metabolism of the animal when lying, thus partially but not wholly compensating for the addition to the metabolism when standing consequent on mastication.

It would appear, then, that the mastication of the heavier rations would tend to increase the ratio of heat production of the animal when standing to that when lying. The heat *elimination*, however, in our experiments showed no distinct evidence of such an increase. The rate of heat emission per minute while the feed was being eaten showed infrequently a slight rise, which was seldom sharp and which was far less than would correspond to the presumable increase in the gaseous exchange. In many instances no effect upon the heat elimination was observed, but rather frequently there was a distinct *fall*. Sometimes, although not in the majority of cases, a rise was observed after the animal had finished eating. The animal was watered after the 6 a. m. feeding. Sometimes no perceptible change in the rate of heat emission resulted, but not infrequently a fall was observed, which was occasionally considerable. It can hardly be doubted that there must have been an increased production of heat during mastication, but apparently this heat was not given off promptly. Part of it at least, it may be conjectured, was applied to warm

the ingesta, especially the water, being eliminated only gradually during the succeeding 12 hours and in part during the periods of lying as well as of standing. It does not seem probable, therefore, that the heat produced in the mastication of the feed was an important factor in causing the differences between the heat elimination of the animals when standing or lying which were observed in these experiments.

Another plausible suggestion seems to be a tendency of the animals to greater restlessness and muscular activity in the standing position when consuming the heavier rations. We are not able to submit direct records to prove this, but indirect evidence is afforded by the fact that the animals as a rule (31 cases out of 40) changed from the lying to the standing position, and vice versa, more frequently on the heavier rations. No such distinct effect was noticeable on the percentage of time spent standing, it being greater in 22 cases and less in 19 on the heavier as compared with the lighter rations and showing a considerable degree of constancy in the individual animal.

INDIVIDUAL DIFFERENCES

In those cases where comparisons between different animals are possible the average results, especially those obtained by comparing the maximum and minimum rations, seem to indicate the existence of distinct individual differences between different animals in this respect.

The most noticeable case of this sort is that of animals A and B, in experiments 190, 200, and 207, steer A being a typical beef animal, while B, although of mixed blood (scrub), was quite distinctly of the dairy type and of a more nervous temperament. In five cases out of six the heat increment due to standing was greater with steer B than with steer A, the average of all the results being 39 per cent higher for the former, as the following summary shows:

*Heat increments in Calories per kilogram of dry matter due to standing*

| Feed. | Steer A. | Steer B. |
|---|---|---|
| Timothy hay | −88 | 148 |
| | 442 | 346 |
| | 364 | 628 |
| Timothy hay and wheat bran | 168 | 200 |
| Timothy hay and grain mixture No. 1 | 600 | 642 |
| | 610 | 828 |
| Average | 335 | 465 |

A similar instance is afforded in experiments 208 and 209 by the animals C, E, and F. Steer C had received almost from birth as heavy feeding as practicable, while E and F had received the same feeds in quantities sufficient to insure normal growth but not to cause any material fattening. The heat increments per kilogram of dry matter of feed were as follows:

*Heat increments in Calories per kilogram of dry matter due to standing*

| Feed. | Steer C. | Steer E. | Steer F. |
|---|---|---|---|
| Alfalfa hay | 118 | 230 | 304 |
| Alfalfa hay and grain mixture No. 2 | −110 | 110 | 144 |
| Average | 4 | 170 | 224 |

INFLUENCE OF BULK OF RATION

It seems worth while to call attention also to three cases which suggest that the bulk of the ration may be a factor in determining the difference between the metabolism of the animals when standing and lying. In period 3 of experiments 208 and 209 steers C, E, and F received a light ration, two-thirds of which consisted of grain—i. e., a ration of small bulk—and on this light ration they showed a distinctly greater increment of metabolism during standing than upon one considerably heavier. It seems at least a plausible suggestion that the deficient bulk may have caused a greater degree of restlessness in period 3 and consequently an increased metabolism.

CORRECTION TO UNIFORM STANDING

Since standing or lying exerts such a marked influence on the heat production of animals, it is evident that a correction for this influence must be made before the results of the heat determinations can be regarded as comparable, since, notwithstanding the uniformity of external conditions striven after, the proportion of time spent standing or lying, respectively, varied more or less. As already pointed out this does not seem to have been related to the quantity of feed consumed, the difference having been practically as often in one direction as in the other. The stimulating effect, if such there were, seems to have expressed itself in more frequent changes of posture and a greater intensity of metabolism while standing rather than in more prolonged standing. The percentage of time spent standing appears to be largely a matter of individuality, whatever that convenient term may really signify. If we compare the results in this respect in the several periods, irrespective of the amount and kind of feed, it appears that, with a few exceptions, they show, on the whole, a rather marked degree of uniformity in the individual animal. This is especially true if experiment 190 be excepted, in which the animals were only about 12 months old. The data are contained in Table VIII, the averages in each case being computed, excepting the bracketed numbers.

TABLE VIII.—*Percentage of time spent standing*

| Animal and experiment No. | Period No. | Percentage of time spent standing. |
|---|---|---|
| Steer I: | | |
| 174 | A | 57 |
| | B | 68 |
| | C | 57 |
| | D | 60 |
| 179 | 1 | 61 |
| | 2 | 63 |
| | 3 | 56 |
| | 4 | [76] |
| 186 | 1a | [87] |
| | 2a | 68 |
| | 3a | 62 |
| Average | | 65 |
| 186 | 1b | 100 |
| | 2b | 81 |
| | 3b | 73 |
| Average | | 85 |
| Steer A: | | |
| 190 | 1 | 79 |
| | 2 | 79 |
| | 3 | 75 |
| | 4 | 81 |
| Average | | 79 |
| 200 | 1 | 52 |
| | 2 | 58 |
| | 3 | 57 |
| | 4 | 51 |
| 207 | 1 | 50 |
| | 2 | 64 |
| | 3 | 46 |
| | 4 | 49 |
| Average | | 53 |
| Steer B: | | |
| 190 | 1 | 65 |
| | 2 | 46 |
| | 3 | 58 |
| | 4 | 28 |
| 200 | 1 | 47 |
| | 2 | 45 |
| | 3 | 57 |
| | 4 | 33 |
| 207 | 1 | 48 |
| | 2 | 46 |
| | 3 | 43 |
| | 4 | 39 |
| Average | | 46 |
| Steer C: | | |
| 208 | 2 | [60] |
| | 3 | 42 |
| | 4 | 47 |
| | 5 | 41 |
| | 6 | 42 |
| Average | | 43 |
| Steer D: | | |
| 208 | 1 | 32 |
| | 2 | 30 |
| 210 | 1 | [53] |
| | 2 | 38 |
| | 3 | 36 |
| 211 | 1 | 30 |
| | 2 | 40 |
| | 3 | 24 |
| | 4 | 36 |
| | 5 | 40 |
| Average | | 34 |
| Steer E: | | |
| 208 | 1 | 30 |
| | 2 | 27 |
| | 3 | 39 |
| | 4 | 40 |
| | 5 | 46 |
| | 6 | [56] |
| Average | | 36 |
| Steer F: | | |
| 209 | 1 | 27 |
| | 2 | 33 |
| | 3 | 32 |
| | 4 | 34 |
| | 5 | 33 |
| | 6 | 46 |
| Average | | 34 |
| Steer G: | | |
| 211 | 1 | 40 |
| | 2 | 42 |
| | 3 | [28] |
| | 4 | 35 |
| | 5 | 30 |
| Average | | 37 |
| Steer H: | | |
| 212 | 1 | 42 |
| | 2 | 40 |
| | 3 | [29] |
| | 4 | 38 |
| | 5 | 34 |
| | 6 | 37 |
| Average | | 38 |

In view of this general uniformity, only a comparatively small correction would be necessary in most instances to make the results on an individual animal comparable as regards standing and lying. When, however, it is desired to compare the results obtained with different individuals so as to get a general average, it is clear that the animal which tends to stand most is at a disadvantage and will show a lower net energy value for the same ration not because his feed is any poorer but because the animal is a less efficient converter and that the greater the stimulus to metabolism exerted by standing the greater will this difference become. It is necessary, therefore, to correct the results upon heat production as well as possible to a uniform proportion of standing and lying, so as to render the results applicable to an (assumed) average animal. According to Table VIII the average percentage of time spent standing was 43. In correcting the results, however, we have used 50 per cent as the standard for convenience in calculation—that is, we have taken as the corrected heat production the average of that standing and lying, computed as shown on page 454 for the entire 48 hours.[1]

## OTHER FORMS OF MUSCULAR ACTIVITY

According to our interpretation of our results, the material increase in the heat production of an animal when standing as compared with that when lying is simply an instance of the well-known influence of muscular exertion upon metabolism. Recent investigators, notably Schlossmann and Murschhauser (42, 43) and Benedict and Talbot (14, 15) in experiments upon infants, and Benedict and Homans (12, 13) in similar trials with dogs, have emphasized the disturbing influence of this factor upon comparisons of different periods. Benedict and his associates, in particular, have devised ingenious methods for determining the degree of muscular activity of a subject and have insisted that only periods of minimum activity can be safely compared.

We have not yet had the courage to attempt to apply to an animal weighing 1,000 or 1,200 pounds methods like those which have been used so successfully for infants and small dogs, either for the indication of minor movements or for the determination of the pulse rate. It seems likely that the latter, in particular, might be of considerable aid in the interpretation of the results if it should prove possible to devise a form of apparatus which would not be injured by the movements of a heavy animal.

[1] This method of correcting the results assumes that the relative intensity of the metabolism of the animal when standing or lying is not affected by the proportion of time spent standing. It might be imagined, however, that in a comparatively long period of standing the original stimulus to incidental muscular movements might gradually fade out, so that the average difference in the rate of metabolism in the two positions would be less for long than for short periods. In this case our method of correcting the results would be more or less erroneous. This possibility can be tested to a certain extent by comparing with each other the two single days of each period. Out of the 64 possible comparisons, that one of the two days in which the lesser percentage of time was spent standing showed a greater increment of standing over lying in 26 cases and a less increment in 37 cases. On the average of the 64 days of maximum standing the percentage of time spent standing was 51.9 and on the 64 days of minimum standing 43.5, while the corresponding average percentage increases in metabolism during standing were, respectively, 41.98 and 41.67. It does not appear, therefore, that within the range of these experiments the ratio of the metabolism when standing to that when lying was materially affected by the proportion of time spent standing.

In considering, however, the weight to be attached to the influence of variations in the muscular activity of the animal, and the degree of refinement necessary in experimental methods, it is important not to forget the main purpose of the experiments. This purpose was, as already explained, substantially an economic one—viz, to determine how much of the energy supplied in metabolizable form by the feed is, under ordinary conditions, dissipated through the heat production caused directly or indirectly by the ingestion of the feed and what proportion of it remains available for the physiological uses of the body. From this point of view it is immaterial whether the increased heat production is caused by "work of digestion" in the narrower sense, by the stimulating effect of the resorbed products of digestion upon the cell metabolism shown by the investigations of Lusk (31, 32, 33, 34, 35, 36, 49), or indirectly by giving rise to increased activity of the voluntary muscles. While it is of interest and value to learn as much as possible of the relative importance of these factors, nevertheless they are "all in the day's work," and from the economic viewpoint their aggregate constitutes the increased energy expenditure consequent upon feed consumption. Even if it were practicable to base comparisons upon periods of minimum activity the results, however interesting physiologically, would include only a part of the effects which the feed actually exerts upon the metabolism. If the feed causes greater restlessness in the animal while standing or causes it to get up and to lie down more frequently, this gives rise, under the conditions of practice, to just as real losses of energy as does the increase of the general cell metabolism when in the lying position and from the economic point of view must be taken into account. What is needed is a comparison of periods of *average* rather than of minimum muscular activity and the correction to 12 hours of standing aims to reduce conditions to such an average (assumed) as regards this very important factor.

Of course, however, the possibility of variations in other forms of muscular activity, arising from differences in external conditions other than the feed, has to be reckoned with. Naturally the endeavor has been to make those conditions as nearly uniform as possible. The feeding was identical from day to day during the three weeks of each period and was given at the same hours. The surroundings during the days spent outside the calorimeter were uniform, and the animals were handled by the same attendants.

During the days in the calorimeter even greater uniformity of conditions existed. The temperature varied only a few hundredths of a degree, the triple walls of the apparatus practically shut off all external sounds except the slight monotonous click of the meter pump, while in the comparatively dim interior the change outside from daylight to artificial light could not have been very noticeable. Visitors were not admitted during the runs. As already stated, all the animals were docile

and accustomed to being handled, to the wearing of the apparatus for the collection of excreta, and to the presence of the observers.

Some idea as to the extent to which these precautions were successful may be formed from a comparison of the quantities of heat produced after correction to 12 hours standing on the two successive days of each 48-hour calorimeter run. The first half of Table IX shows the corrected heat production on the first and second days of each run and likewise the mean, computed in the manner illustrated on page 454 for the entire 48 hours.[a]

TABLE IX.—*Heat production per day and per head corrected to 12 hours standing*

| Feeding stuff and experiment No. | Animal No. | Period No. | Corrected heat production. | | | Analysis of heat production. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | First day. | Second day. | 48-hour mean. | Standing 12 hours. | Rising and lying down. | $CH_4$ fermentation. | Remainder. |
| Timothy hay: | | | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* |
| 174 | I | A | 8,788 | 9,316 | 9,049 | 1,112 | 17 | 448 | 7,472 |
| | I | B | 9,899 | 9,565 | 9,769 | 1,450 | 18 | 570 | 7,731 |
| | I | C | 10,309 | 10,723 | 10,562 | 1,440 | 17 | 626 | 8,479 |
| | I | D | 11,013 | 11,130 | 11,149 | 1,437 | 27 | 864 | 8,821 |
| 190 | A | 3 | 5,709 | 5,532 | 5,636 | 1,091 | 12 | 282 | 4,251 |
| | A | 4 | ?7,535 | 6,545 | 6,709 | 1,023 | 15 | 481 | 5,190 |
| | B | 3 | 4,985 | 4,904 | 5,081 | 875 | 12 | 276 | 3,918 |
| | B | 4 | 6,095 | 5,886 | 5,852 | 937 | 12 | 361 | 4,542 |
| 200 | A | 3 | 7,186 | 7,275 | 7,245 | 1,001 | 25 | [b] 378 | 5,841 |
| | A | 4 | 8,057 | 8,205 | 8,186 | 1,379 | 40 | [b] 606 | 6,161 |
| | B | 3 | 6,773 | 6,707 | 6,778 | 1,093 | 24 | [b] 367 | 5,294 |
| | B | 4 | 7,750 | 7,783 | 7,837 | 1,315 | 33 | [b] 540 | 5,949 |
| 207 | A | 3 | 7,713 | 7,812 | 7,791 | 1,107 | 40 | 498 | 6,146 |
| | A | 4 | 9,267 | 9,649 | 9,523 | 1,438 | 59 | 704 | 7,232 |
| | B | 3 | 7,996 | 8,070 | 8,064 | 1,190 | 43 | 481 | 6,350 |
| | B | 4 | 9,621 | 9,637 | 9,812 | 1,758 | 51 | 747 | 7,256 |
| Red clover hay: | | | | | | | | | |
| 179 | I | 1 | 10,930 | 10,910 | 10,926 | 2,354 | 43 | 626 | 7,903 |
| | I | 2 | 9,327 | 9,944 | 9,621 | 1,812 | 28 | 464 | 7,317 |
| 186 | I | 1a | 9,844 | 9,586 | 9,627 | 1,604 | 20 | 413 | 7,590 |
| | I | 3a | 9,835 | 10,618 | 10,176 | 1,958 | 51 | 404 | 7,703 |
| | I | 2a | 10,335 | 10,945 | 10,574 | 2,183 | 36 | 555 | 7,800 |
| | I | 3b | 10,178 | 10,197 | 10,200 | 1,402 | 48 | 464 | 8,202 |
| | I | 2b | 10,274 | 10,976 | 10,501 | 1,688 | 38 | 555 | 8,220 |
| Mixed hay: | | | | | | | | | |
| 211 | D | 1 | 11,775 | 12,005 | 12,350 | 1,679 | 79 | 805 | 9,706 |
| | D | 4 | 9,481 | 9,680 | 9,625 | 1,498 | 76 | 527 | 7,524 |
| | D | 5 | 8,291 | 8,250 | 8,258 | 1,497 | 57 | 282 | 6,422 |
| 211 | G | 1 | 12,211 | 11,071 | 12,008 | 1,802 | 80 | 830 | 9,386 |
| | G | 4 | 9,843 | 9,242 | 9,568 | 1,958 | 75 | 457 | 7,078 |
| | G | 5 | 7,811 | 7,178 | 7,477 | 1,382 | 62 | 261 | 5,772 |

[a] The 48-hour means differ from the means of the 24 hour periods for two reasons: First, the percentage increase of the metabolism when standing over that when lying varied in the two days, as did also the percentage of the total heat carried off as latent heat of water vapor. On account especially of the latter difference, the mean of the two days taken singly differs from that computed from the average heat production per minute standing and lying for the whole 48 hours. Second, in experiments 190, 200, and 207 the corrected results for the 48 hours are computed from selected portions of the runs in the manner described in an earlier publication (10, p. 43), and therefore differ from the mean of the results for the single days.

[b] Methane computed from digested carbohydrates.

TABLE IX.—*Heat production per day and per head corrected to 12 hours standing*—Con.

| Feeding stuff and experiment No. | Animal No. | Period No. | Corrected heat production. | | | Analysis of heat production. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | First day. | Second day. | 48-hour mean. | Standing 12 hours. | Rising and lying down. | $CH_4$ fermentation. | Remainder. |
| Alfalfa hay: | | | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* |
| | D | 1 | 5,385 | 5,442 | 5,411 | 1,000 | 39 | 256 | 4,116 |
| | D | 2 | 4,243 | 4,290 | 4,266 | 612 | 45 | 161 | 3,448 |
| | E | 4 | 7,931 | 7,823 | 7,879 | 1,014 | 58 | 457 | 6,350 |
| 208 | E | 5 | 5,759 | 5,764 | 5,764 | 796 | 45 | 302 | 4,621 |
| | E | 6 | 4,529 | 4,590 | 4,566 | 732 | 24 | 180 | 3,630 |
| | C | 4 | 8,965 | 9,100 | 9,052 | 1,297 | 48 | 470 | 7,237 |
| | C | 5 | 7,340 | 7,391 | 7,357 | 1,292 | 38 | 380 | 5,647 |
| | C | 6 | 6,136 | 6,122 | 6,124 | 1,156 | 34 | 271 | 4,663 |
| | F | 4 | 11,228 | 11,432 | 11,474 | 1,400 | 86 | 697 | 9,291 |
| 209 | F | 5 | ...... | 8,031 | 8,007 | 948 | 43 | 429 | 6,587 |
| | F | 6 | 6,819 | 6,794 | 6,781 | 830 | 54 | 285 | 5,612 |
| | H | 1 | 11,424 | 11,186 | 11,272 | 1,522 | 62 | 837 | 8,851 |
| 212 | H | 3 | 10,189 | 10,616 | 10,388 | 1,549 | 63 | 685 | 8,091 |
| | H | 5 | 7,836 | 7,582 | 7,754 | 1,196 | 45 | 415 | 6,098 |
| Alfalfa meal: | | | | | | | | | |
| | H | 2 | 11,025 | 11,505 | 11,252 | 1,570 | 53 | 760 | 8,869 |
| 212 | H | 4 | 9,889 | 10,270 | 10,066 | 1,425 | 46 | 638 | 7,957 |
| | H | 6 | 7,027 | 7,132 | 7,069 | 1,146 | 42 | 419 | 5,462 |
| Maize stover: | | | | | | | | | |
| | D | 1 | 9,550 | 9,203 | 9,363 | 1,428 | 31 | 495 | 7,409 |
| 210 | D | 2 | 8,605 | 8,404 | 8,495 | 1,199 | 44 | 405 | 6,847 |
| | D | 3 | 7,527 | 7,429 | 7,476 | 1,250 | 59 | 308 | 5,859 |
| Maize meal added to clover hay: | | | | | | | | | |
| | I | [a]2 | 9,327 | 9,944 | 9,621 | 1,812 | 28 | 464 | 7,317 |
| 179 | I | 3 | 10,483 | 9,876 | 10,198 | 2,284 | 45 | 630 | 7,239 |
| | I | 4 | 12,723 | 13,354 | 12,947 | 3,124 | 21 | 1,110 | 8,689 |
| Wheat bran added to timothy hay: | | | | | | | | | |
| | A | [a]3 | 5,709 | 5,532 | 5,636 | 1,091 | 12 | 282 | 4,251 |
| | A | 1 | 7,819 | 7,328 | 7,739 | 1,486 | 12 | 503 | 5,738 |
| | A | 2 | ...... | 8,400 | 8,385 | 1,307 | 13 | 643 | 6,422 |
| 190 | B | [a]3 | 4,985 | 4,904 | 5,081 | 875 | 12 | 276 | 3,918 |
| | B | 1 | 6,493 | 6,299 | 6,363 | 865 | 6 | 400 | 5,092 |
| | B | 2 | 6,814 | 7,117 | 7,409 | 1,057 | 11 | 537 | 5,804 |
| Grain mixture No. 1 added to timothy hay: | | | | | | | | | |
| | A | [a]3 | 7,186 | 7,275 | 7,245 | 1,001 | 25 | [a]378 | 5,841 |
| | A | 1 | 8,955 | 9,236 | 9,274 | 1,322 | 32 | [a]679 | 7,241 |
| 200 | A | 2 | 12,140 | 12,667 | 12,514 | 2,226 | 48 | [a]1,043 | 9,197 |
| | B | [a]3 | 6,773 | 6,707 | 6,778 | 1,093 | 24 | [a]367 | 5,294 |
| | B | 1 | 8,916 | 8,952 | 9,016 | 1,427 | 17 | [a]556 | 7,016 |
| | B | 2 | 9,810 | 9,710 | 9,909 | 1,771 | 25 | [a]684 | 7,429 |
| | A | [a]3 | 7,713 | 7,812 | 7,791 | 1,107 | 40 | 498 | 6,146 |
| | A | 1 | 9,786 | 10,181 | 10,164 | 1,294 | 58 | 841 | 7,971 |
| | A | 2 | 13,534 | 12,738 | 13,375 | 2,518 | 73 | 1,222 | 9,562 |
| 207 | B | [a]3 | 7,996 | 8,070 | 8,064 | 1,190 | 43 | 481 | 6,350 |
| | B | 1 | 9,434 | 9,568 | 9,600 | 1,558 | 24 | 733 | 7,285 |
| | B | 2 | 11,658 | 11,640 | 11,720 | 2,285 | 45 | 954 | 8,436 |

[a] Basal ration of coarse fodder only.

TABLE IX.—*Heat production per day and per head corrected to 12 hours standing*—Con.

| Feeding stuff and experiment No. | Animal No. | Period No. | Corrected heat production. | | | Analysis of heat production. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | First day. | Second day. | 48-hour mean. | Standing 12 hours. | Rising and lying down. | $CH_4$ fermentation. | Remainder. |
| Alfalfa hay and grain mixture No. 2: | | | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* |
| 208 | E | 1 | 7,434 | 7,532 | 7,483 | 916 | 56 | 464 | 6,047 |
| | E | 2 | 5,940 | 5,911 | 5,924 | 788 | 53 | 303 | 4,780 |
| | E | 3 | 5,032 | 5,138 | 5,084 | 824 | 35 | 165 | 4,060 |
| | C | 2 | 6,802 | 6,927 | 6,858 | 1,011 | 31 | 388 | 5,428 |
| | C | 3 | 6,096 | 6,149 | 6,123 | 1,043 | 34 | 284 | 4,762 |
| 209 | F | 1 | 9,679 | 10,099 | 9,888 | 1,132 | 89 | 562 | 8,105 |
| | F | 2 | 7,645 | 7,809 | 7,115 | 844 | 73 | 382 | 5,816 |
| | F | 3 | 6,791 | 6,659 | 6,734 | 948 | 68 | 253 | 5,465 |
| Mixed hay and maize meal: | | | | | | | | | |
| 211 | G | 2 | 8,678 | 8,362 | 8,470 | 1,505 | 74 | 470 | 6,421 |
| | G | 3 | 14,510 | 14,467 | 14,561 | 2,852 | 68 | 1,126 | 10,515 |
| Mixed hay and hominy chop: | | | | | | | | | |
| 211 | D | 2 | 9,782 | 10,126 | 9,947 | 1,695 | 71 | 643 | 7,538 |
| | D | 3 | 14,877 | 15,040 | 14,936 | 1,850 | 102 | 1,216 | 11,768 |

As already stated, we estimate the experimental error in the determination of the heat emitted by the animal to be approximately 1 per cent. In the 73 cases in which a comparison of the two days can be made, 40 show a deviation from the mean of the two 24-hour results of less than 1.1 per cent—i. e., the results for the two days practically agree within the limits of experimental error. Of the 53 experiments made since 1905—i. e., experiments 200 to 212—which, in our judgment, are, on the whole, more accurate than the earlier ones, 36 fall within this limit or error. On the other hand, however, deviations as great as 2 per cent are not uncommon, while occasionally they rise to as much as 5 per cent or even 7 per cent. The mean of the percentage deviations is for the entire series 1.45 per cent and for experiments 200 to 212, inclusive, 1.13 per cent. Moreover, the deviations of the single days from the mean are, on the whole, fully as great after reduction to 12 hours standing as before. It is clear, therefore, that despite the apparent uniformity of experimental conditions the metabolism of the animals was affected by influences other than the feed or the proportion of time spent standing.

Mr. H. H. Mitchell, of the Illinois Experiment Station, has had the kindness to submit these data to mathematical study and finds that they present clear evidence of the existence of individual differences between the animals as regards the agreement between the two days. He writes as follows:

It seems very evident to me both from inspection of the data and from statistical calculations that the percentage deviation of duplicate determinations of the heat

production of animals is variable, depending undoubtedly upon the particular animal under investigation and possibly, also, upon the nature of the ration. Thus the average percentage deviations for the four animals for which 10 or more observations are recorded, with their probable errors, are:

| | |
|---|---|
| Animal I | 2.10±0.23 |
| Animal A | [a] 2.25±0.35 |
| Animal A | [b] 1.77±0.18 |
| Animal B | 0.75±0.13 |
| Animal D | 1.32±0.28 |

While, according to these figures, animals I and A can not be differentiated from each other, they are both clearly differentiated from animal B, the differences, with their probable errors, being 1.35±0.26 between animals I and B and 1.02±0.22 between animals A and B. The difference between animals I and D was 0.78±0.36, the significance of which may be questioned. Using another method of comparison, it may be shown that the odds are 124 to 1 that animals C and H are definitely distinct as regards the percentage deviation under discussion. I should not hesitate to conclude, therefore, that this percentage deviation is affected by the individuality of the experimental animals. Furthermore, there is a slight suggestion, especially in the data of animals A and B, that the nature of the ration may affect the percentage deviation of your determinations.

It is of interest to note in this connection that the results of Kellner's respiration experiments (uncorrected for standing or lying) likewise show variations of much the same order of magnitude between individual (not consecutive) days. When, therefore, comparisons are based upon the average results for 48 hours, it is impossible to assert that these results represent, as they should, periods of average muscular activity, although it would appear that the error thus introduced is usually not large. In Kellner's experiments it is still further reduced by the fact that in most cases the results of four or five single runs are averaged.

## ANALYSIS OF HEAT PRODUCTION

In Table VII were shown the increments of heat production per 24 hours in standing animals as compared with those lying. It is evident that of the total corrected heat production recorded in Table IX an amount equal to one-half of the corresponding increment shown by Table VII is to be regarded as the effect of the 12 hours' standing, while the remainder represents the metabolism of the animal per 24 hours lying. On the basis of Zuntz's recent results it is possible to carry this analysis of the heat production a little farther, at least approximately. The expenditure of energy caused by standing obviously includes that required for the muscular effort of rising and lying down. Von der Heide, Klein, and Zuntz (20, p. 823) estimate this on the basis of experiments by Klein at 9.7 Calories per 550 kg. of live weight for once rising and lying down again. The same investigators (20, p. 705) compute from Markoff's experiments (37, 38) that the methane fermentation in cattle gives rise to the evolution of 4.374 Calories of heat per cubic centimeter of methane, equivalent to 6.07 Calories per gram. While both the foregoing figures are confessedly but approximations, nevertheless they permit a partial analysis of the heat production with

[a] Including questionable observation. [b] Not including questionable observation.

the results shown in the second part of Table IX. The "remainder" shown in the last column includes the so-called basal or fasting metabolism, together with the effect of the feed in increasing the muscular activity of the organs of digestion and of the voluntary muscles in the lying position, as well as in directly stimulating the cell metabolism. No sufficient data are available for further analysis of this "remainder."

## PROPORTION OF FEED ENERGY EXPENDED IN HEAT PRODUCTION

### METHOD OF DETERMINATION

The total metabolism of an animal upon any particular ration, as illustrated by the figures of Table IX, is made up of numerous factors, and a single experiment affords no means of determining the proportion due to the consumption of feed. This can be determined only by a comparison of two periods, otherwise identical, in which different quantities of the same feed are consumed, the additional heat production on the heavier ration constituting the measure of the additional energy expended. With carnivora and with man the comparison may be made with the fasting state—i. e., the amount of feed in one of the periods may be zero. With cattle this is impracticable for obvious reasons, and it is necessary to make the comparison with a period upon a so-called basal ration. Kühn and Kellner added the feeding stuffs to be tested to a mixed basal ration that was more than sufficient for maintenance. In our earlier tests, up to experiment 207, inclusive, the same general plan was followed, except that the basal ration consisted of coarse feeds only and was in most cases below the maintenance requirement. In the later experiments the method was modified by feeding different quantities of the same feed or mixture of feeds both above and below the maintenance requirement. The method of comparison for a ration consisting of a single feeding stuff is very simple. Thus, in experiment 207 the following results were obtained on timothy hay with steer A in periods 3 and 4. The same method of comparison may obviously be applied also to different amounts of a mixed ration of grain and hay.

*Computation of energy expenditure by steer A per kilogram of timothy hay eaten*

| Item. | Quantity of dry matter eaten. | Total heat production. | Distribution of heat production. | | | |
|---|---|---|---|---|---|---|
| | | | Standing. | Rising and lying down. | Fermentation. | Remainder. |
| | *Gm.* | *Calories.* | *Calories.* | *Calories.* | *Calories.* | *Calories.* |
| Period 4 | 4,892 | 9,523 | 1,438 | 59 | 794 | 7,232 |
| Period 3 | 2,974 | 7,791 | 1,107 | 40 | 408 | 6,146 |
| Difference | 1,918 | 1,732 | 331 | 19 | 296 | 1,086 |
| Difference per kilogram of dry matter | | 903 | 173 | 9 | 154 | 567 |

The comparison of these two periods shows that each additional kilogram of dry matter consumed increased the total heat production by 903 Calories, 173 of which represent energy expended in standing, 9 that expended in rising and lying down, and 154 the additional heat due to the methane fermentation, while the remainder, 567 Calories, represents the increased mechanical work of digestion plus any stimulus which the digested nutrients exerted upon the cell metabolism. Obviously the calculation by difference eliminates the basal metabolism.

For concentrated feeds, which can not be fed alone, two methods have been used, as already noted. In the earlier experiments, the concentrate was added to the basal ration of coarse fodder. Thus, in period 2 of the experiment just used as an illustration a mixture of grains (grain mixture No. 2) was added to the basal ration of period 3 with the following results:

*Computation of energy expenditure by steer A per kilogram of grain eaten*

| Item. | Quantity of dry matter eaten. | | Total heat production. | Distribution of heat production. | | | |
|---|---|---|---|---|---|---|---|
| | Hay. | Grain. | | Standing. | Rising and lying down. | Fermentation. | Remainder. |
| | *Gm.* | *Gm.* | *Calories.* | *Calories.* | *Calories.* | *Calories.* | *Calories.* |
| Period 2 | 2,949 | 4,759 | 13,375 | 2,518 | 73 | 1,222 | 9,562 |
| Period 3 | 2,974 | ........ | 7,791 | 1,107 | 40 | 498 | 6,146 |
| Difference | −25 | 4,759 | 5,584 | 1,411 | 33 | 724 | 3,416 |
| Difference per kilogram of dry matter | ........ | ........ | 1,179 | 298 | 7 | 153 | 721 |

Each kilogram of dry matter of the grain increased the heat production by 1,179 Calories, which can be subdivided as before in the proportions shown.[1] The greater expenditure of energy per kilogram in the case of grain as compared with hay is seen to be due in part to a greater increase of the metabolism of the animal when standing and in part either to increased mechanical work in digestion or more likely to a greater stimulus of the cell metabolism.

In later experiments (Nos. 208 to 212, inclusive), in place of adding grain to a ration of coarse fodder, the animals received varying quantities of a uniform mixture of coarse fodder and grain, the energy expenditure caused by the total ration being determined substantially in the manner already illustrated. The portion of the increase due to the grain alone was computed by subtracting from the total increase that due to the hay as determined in two or more separate periods on exclusive hay rations.[2] The method may be illustrated by the results obtained with steer E in periods 1 and 3 of experiment 208.

[1] Logically the results of the comparison should be corrected for the slight difference (25 gm.) in the amount of dry matter of hay consumed. As a matter of fact, however, this correction is insignificant in all the experiments, amounting in the present instance to about 1 Calorie.

[2] When more than two periods of hay feeding were used the increased heat production per kilogram of hay was computed by comparing the periods on the heaviest and the lightest rations.

*Computation of energy expenditure of steer E per kilogram of grain eaten*

| Item. | Quantity of dry matter eaten. | | Total heat production. | Distribution of heat production. | | | |
|---|---|---|---|---|---|---|---|
| | Hay. | Grain. | | Standing. | Rising and lying down. | Fermentation. | Remainder. |
| | *Gm.* | *Gm.* | *Calories.* | *Calories.* | *Calories.* | *Calories.* | *Calories.* |
| Period 1 | 1,086 | 2,122 | 7,483 | 916 | 56 | 464 | 6,047 |
| Period 3 | 387 | 764 | 5,084 | 824 | 35 | 165 | 4,060 |
| Difference | 699 | 1,358 | 2,399 | 92 | 21 | 299 | 1,987 |
| Difference due to 699 gm. of hay [a] | | | 840 | 71 | 9 | 70 | 690 |
| Difference due to 1,358 gm. of grain | | | 1,559 | 21 | 12 | 229 | 1,297 |
| Difference per kilogram of grain | | | 1,148 | 15 | 9 | 169 | 955 |

[a] Computed from a comparison of periods 4 and 6.

## DIFFERENCES IN LIVE WEIGHT

As the figures of Table VII show, the live weight of the animals varied more or less in the different periods. To what extent do these variations affect the conclusions drawn from comparisons like those just illustrated?

Two effects other than those due directly to the amount of feed might be anticipated from an increase in the live weight: First, an increase in the basal metabolism due to a greater mass of tissue, and, second, an increase in the muscular work of supporting the body in the standing position. As regards the first of these, it is to be remarked that the experimental periods were short (three or, in a few cases, four weeks only) while the changes in the amount and kind of feed consumed were considerable. It seems altogether probable that the larger part of the variation in weight must be ascribed to "fill"—i. e., to variations in the contents of the digestive tract rather than to any considerable change in the make-up of the body proper—and that the actual basal metabolism was not greatly affected. As regards the effect upon the muscular work of standing, it has been already pointed out that this appears to be a relatively small factor in the total increase of heat elimination in standing. In view of these considerations, it is to be anticipated that a correction of the heat production in proportion to either the weight or the surface of the animals would materially exaggerate the effect upon the metabolism, and, on the whole, we have regarded it as safer to disregard the variations in live weight rather than to attempt a more or less conjectural correction.

HEAT INCREMENTS PER KILOGRAM OF DRY MATTER

The results of the comparisons between periods made by the methods just illustrated are contained in Table X. In those cases in which more than two periods upon the same ration can be compared, the total heat increments per kilogram of dry matter are recorded for each successive

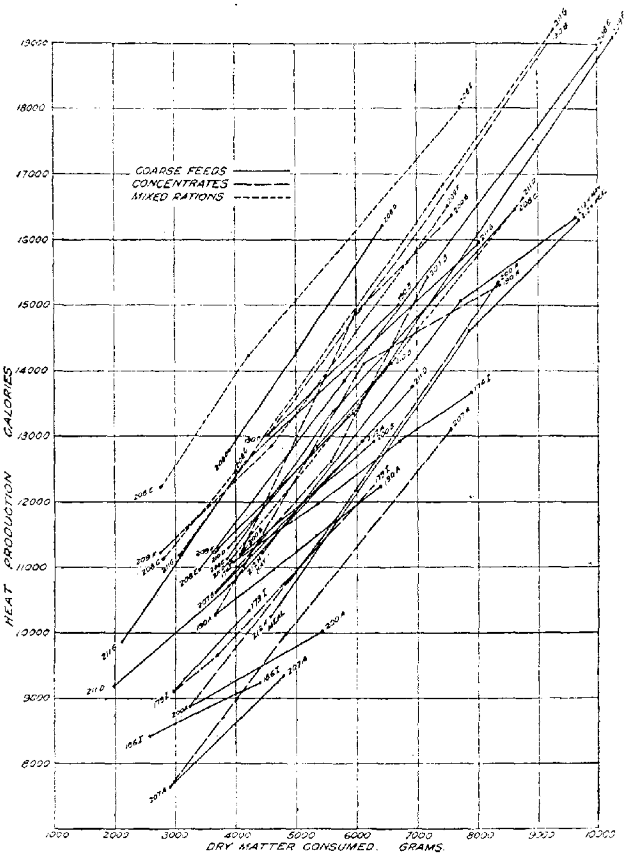


FIG. 2.—Graph showing the relation of heat production to dry matter consumed; computed per 500 kg. live weight.

pair of periods, beginning with the lighter ration. Thus, in the case of steer I in experiment 174 four different amounts of timothy hay[1] were eaten. Table X shows the total heat increments computed by comparing periods A with B; B with C; C with D; and finally A with D. The

[1] About 300 gm. of linseed meal in each period were also fed.

final comparison, between the smallest and greatest rations, is regarded as the average result, and on it is based the computation of the distribution of this energy between standing, rising, and lying down, fermentation, and the "remainder," as well as, in the case of mixed rations, the computation of the energy increment due to the hay. The same results, computed per 500 kg. of live weight, are also represented graphically in figure 2, the abscissæ representing the amount of dry matter consumed and the ordinates the corresponding corrected heat production, the heat production per kilogram of dry matter corresponding to the tangent of the angle between the graph and the horizontal axis.

TABLE X.—*Increments of heat production per kilogram of dry matter*

| Feeding stuff and experiment No. | Animal No. | Successive amounts of feed. | | | Comparison of greatest and least amounts of feed. | Analysis of heat increments. Standing 12 hours. | Rising and lying down. | $CH_4$ fermentation. | Remainder. |
|---|---|---|---|---|---|---|---|---|---|
| | | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* |
| Timothy hay: | | | | | | | | | |
| 174 | I | 634 | 716 | 612 | 656 | 102 | 3 | 130 | 421 |
| 190 | A | | | | 719 | −46 | 2 | 133 | 630 |
| | B | | | | 922 | 74 | 0 | 102 | 746 |
| 200 | A | | | | 529 | 213 | 8 | 128 | 180 |
| | B | | | | 793 | 166 | 7 | 130 | 490 |
| 207 | A | | | | 903 | 173 | 9 | 154 | 567 |
| | B | | | | 954 | 310 | 4 | 145 | 495 |
| Average | | | | | 782 | 141 | 5 | 132 | 504 |
| Red clover hay: | | | | | | | | | |
| 179 | I | | | | 992 | 412 | 11 | 123 | 446 |
| 186 | I, series *a* | 455 | 449 | | 453 | 277 | 8 | 68 | 100 |
| Average | | | | | 723 | 344 | 10 | 96 | 273 |
| 186 | I, series *b* | | | | 333 | 220 | −10 | 103 | 20 |
| Mixed hay: | | | | | | | | | |
| 211 | D | 799 | 1,010 | | 928 | 41 | 5 | 118 | 764 |
| | G | 1,357 | 860 | | 1,031 | 94 | 4 | 137 | 806 |
| Average | | 1,078 | 935 | | 980 | 67 | 5 | 123 | 785 |
| Alfalfa hay: | | | | | | | | | |
| 208 | D | | | | 1,339 | 454 | −7 | 111 | 781 |
| | E | 1,204 | 1,200 | | 1,202 | 102 | 13 | 100 | 987 |
| | C | 1,200 | 1,030 | | 1,110 | 54 | 5 | 70 | 981 |
| 209 | F | 918 | 1,327 | | 1,189 | 145 | 8 | 104 | 932 |
| 212 | H | 1,161 | 671 | | 981 | 91 | 5 | 118 | 767 |
| Average | | 1,147 | 1,051 | | 1,165 | 169 | 5 | 102 | 889 |

TABLE X.—*Increments of heat production per kilogram of dry matter*—Continued

| Feeding stuff and experiment No. | Animal No. | Successive amounts of feed. | | | Comparison of greatest and least amounts of feed. | Analysis of heat increments. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Standing 12 hours. | Rising and lying down. | $CH_4$ fermentation. | Remainder. |
| | | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* |
| Alfalfa meal: | | | | | | | | | |
| 212 | H | 1,330 | 939 | ...... | 1,190 | 121 | 3 | 97 | 969 |
| Average of alfalfa hay and meal | .......... | 1,184 | 1,028 | ...... | 1,169 | 161 | 5 | 101 | 902 |
| Maize stover: | | | | | | | | | |
| 210 | D | 1,034 | 1,101 | ...... | 1,065 | 101 | −16 | 105 | 875 |
| Alfalfa hay and grain mixture No. 2: | | | | | | | | | |
| 208 | E | 1,395 | 1,072 | ...... | 1,166 | 45 | 10 | 145 | 966 |
| | C | ...... | ...... | ...... | 1,139 | −50 | −5 | 161 | 1,033 |
| 209 | F | 886 | 1,244 | ...... | 1,105 | 64 | 7 | 108 | 926 |
| Average | .......... | 1,146 | 1,160 | ...... | 1,139 | 20 | 4 | 138 | 977 |
| Alfalfa hay and grain mixture No. 2 (periods 1 and 2): | | | | | | | | | |
| 208 | E | ...... | ...... | ...... | 1,072 | 88 | 2 | 111 | 871 |
| | C | ...... | ...... | ...... | ...... | ...... | ...... | ...... | ...... |
| 209 | F | ...... | ...... | ...... | 1,248 | 169 | 9 | 103 | 971 |
| Average | .......... | ...... | ...... | ...... | 1,160 | 127 | 5 | 107 | 921 |
| Mixed hay and maize meal: | | | | | | | | | |
| 211 | G | ...... | ...... | ...... | 1,297 | 287 | −1 | 140 | 871 |
| Mixed hay and hominy chop: | | | | | | | | | |
| 211 | D | ...... | ...... | ...... | 1,147 | 36 | 7 | 132 | 972 |
| Maize meal added to clover hay: | | | | | | | | | |
| 179 | I | 765 | 1,004 | ...... | 952 | 375 | −1 | 185 | 393 |
| Maize meal (computed): | | | | | | | | | |
| 211 | G | ...... | ...... | ...... | 1,434 | 386 | −4 | 146 | 906 |
| Hominy chop (computed): | | | | | | | | | |
| 211 | D | ...... | ...... | ...... | 1,365 | 30 | 9 | 146 | 1,180 |
| Wheat bran added to timothy hay: | | | | | | | | | |
| 190 | A | 1,561 | 525 | ...... | 1,066 | 84 | 0 | 140 | 842 |
| | B | 1,246 | 1,343 | ...... | 1,288 | 101 | −1 | 144 | 1,044 |
| Average | .......... | 1,404 | 934 | ...... | 1,177 | 93 | −1 | 142 | 943 |

TABLE X.—*Increments of heat production per kilogram of dry matter*—Continued

| Feeding stuff and experiment No. | Animal No. | Successive amounts of feed. | | | Comparison of greatest and least amounts of feed. | Analysis of heat increments. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Standing 12 hours. | Rising and lying down. | $CH_4$ fermentation. | Remainder. |
| Grain mixture No. 1 added to timothy hay: | | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* |
| 200 | A | 1,157 | 1,348 | ..... | 1,267 | 294 | 6 | 160 | 807 |
| | B | 1,933 | 935 | ..... | 1,482 | 321 | 0 | 150 | 1,011 |
| 207 | A | 1,213 | 1,156 | ..... | 1,179 | 298 | 7 | 153 | 721 |
| | B | 1,129 | 1,640 | ..... | 1,378 | 413 | 1 | 178 | 786 |
| Average | | 1,358 | 1,270 | ..... | 1,327 | 331 | 4 | 161 | 831 |
| Grain mixture No. 2 (computed): | | | | | | | | | |
| 208 | E | 1,494 | 1,004 | ..... | 1,148 | 15 | 9 | 169 | 955 |
| | C | ..... | ..... | ..... | 1,152 | −99 | −9 | 202 | 1,058 |
| 209 | F | 759 | 1,277 | ..... | 1,077 | 27 | 7 | 110 | 933 |
| Average | | 1,127 | 1,141 | ..... | 1,125 | −19 | 2 | 160 | 982 |
| Grain mixture No. 2 (computed from periods 1 and 2): | | | | | | | | | |
| 208 | E | ..... | ..... | ..... | 1,004 | 80 | −3 | 116 | 811 |
| 209 | F | ..... | ..... | ..... | 1,277 | 175 | 9 | 103 | 990 |
| Average | | ..... | ..... | ..... | 1,141 | 128 | 3 | 110 | 900 |

## CRITICAL TEMPERATURE

In order that comparisons like the foregoing shall be valid, the experiments must, of course, be made above the so-called "critical temperature" for the animal experimented with and for the minimum quantity of feed consumed, since below this temperature part of the heat produced is utilized to maintain the body temperature and thus to reduce the amount of heat liberated by the katabolism of body substance (2, p. 347–359, 407–410). Our experiments have been made at about 17° to 18° C., and we have not attempted to determine the critical temperature for cattle, but the fact clearly shown in figure 2 that the heat production per kilogram of feed consumed showed no tendency to increase as the rations were made heavier leads us to believe that even on the lightest rations the temperature was safely above the point at which the so-called "chemical" regulation of body temperature begins. Kellner's experiments were made at somewhat lower temperatures, mostly between 12° and 15° C., but on heavier rations.

DISCREPANCIES IN RESULTS

It is apparent from both Table X and figure 2 that the single results show a considerable range for the same or similar feeds not only with different animals but also, in some instances, between different periods with the same animal. For example, in experiment 209, on steer F with alfalfa hay an increase of the ration from 2,226 to 3,562 gm. of dry matter caused an increase in the (corrected) heat production at the rate of 963 Calories per kilogram, while a further increase to 6,174 gm. resulted in a relatively greater increase of the heat production—viz, 1,301 Calories per kilogram.

In the instance just cited one might be inclined to interpret the difference as an effect of the greater feed consumption. The next line in Table X, however, shows an even greater difference in the opposite direction, while it is evident from figure 2 that the data as a whole show about as many differences in one direction as the other and, as pointed out in the previous paragraph, fail to give any distinct evidence of a greater relative increase of heat production on heavy as compared with light feed or on supermaintenance as compared with submaintenance rations, the averages tending, if anything, to be a trifle lower on the heavier rations.

Unavoidable differences in the muscular activities of the animal, other than those connected with standing and lying, and in other conditions have also to be considered. As already pointed out, the existence of such differences, in spite of the uniformity of the controllable experimental conditions, is indicated by the occasionally considerable divergence of the heat production upon the two days of the calorimeter runs. It is not improbable, therefore, that they may be responsible, at least in part, for the observed discrepancies, so that it is obvious that the average results must be accepted with some reserve. On the other hand, however, it must be remembered that these are calculations by difference and that in such a calculation the experimental errors tend to accumulate in the final result. Obviously the greater we make the difference in the factor whose effect is to be determined, the less will be the relative error of the final result.[1] We believe, therefore, that the results obtained by a comparison of the extreme rations, as recorded in column 4 of Table X, are decidedly more trustworthy than those computed from the intermediate rations, and, notwithstanding the discrepancies just mentioned, are inclined to regard them as expressing the total effect of the feed in increasing the metabolism, when variations in the time of standing are eliminated, with a sufficient degree of accuracy to warrant general comparisons of the average results. These average results, both as to the total heat increment and its factors, are summarized in Table XI.

[1] Out of 13 cases in which the results appear abnormally high or low, there were 6 in which the difference in dry matter consumed was less than 1 kg., although there were 5 other cases in which, with a similar small difference in the dry matter consumed, apparently normal results were obtained.

TABLE XI.—*Average increments of heat production per kilogram of dry matter*

| Feeding stuff. | Total increment. | Analysis of heat increment. | | | |
|---|---|---|---|---|---|
| | | Standing 12 hours. | Rising and lying down. | $CH_4$ fermentation. | Remainder. |
| COARSE FODDERS: | *Cals.* | *Cals.* | *Cals.* | *Cals.* | *Cals.* |
| Timothy hay | 782 | 141 | 5 | 132 | 504 |
| Red clover hay: | | | | | |
| Average | 723 | 344 | 10 | 96 | 273 |
| Experiment 179 | 992 | 412 | 11 | 123 | 446 |
| Mixed hay | 980 | 68 | 4 | 123 | 785 |
| Alfalfa hay | 1,165 | 169 | 5 | 102 | 889 |
| Alfalfa meal | 1,190 | 121 | 3 | 97 | 969 |
| Average of alfalfa hay and meal | 1,169 | 161 | 5 | 101 | 902 |
| Maize stover | 1,065 | 101 | −16 | 105 | 875 |
| MIXED RATIONS: | | | | | |
| Alfalfa hay and grain mixture No. 2 | | | | | |
| Average of all | 1,139 | 20 | 4 | 138 | 977 |
| Average of periods 1 and 2 only | 1,160 | 127 | 5 | 107 | 921 |
| Mixed hay and maize meal | 1,297 | 287 | − 1 | 140 | 871 |
| Mixed hay and hominy chop | 1,147 | 36 | 7 | 132 | 972 |
| CONCENTRATES: | | | | | |
| Maize meal added to clover hay | 952 | 375 | − 1 | 185 | 393 |
| Maize meal computed from mixed ration | 1,434 | 386 | − 4 | 146 | 906 |
| Hominy chop computed from mixed ration | 1,365 | 30 | 9 | 146 | 1,180 |
| Wheat bran added to timothy hay | 1,177 | 93 | 1 | 142 | 943 |
| Grain mixture No. 1 added to timothy hay | 1,327 | 331 | 4 | 161 | 831 |
| Grain mixture No. 2 computed from mixed ration | 1,125 | −19 | 2 | 160 | 982 |
| The same from periods 1 and 2 only | 1,141 | 128 | 3 | 110 | 900 |

COMPARISON OF COARSE FEEDS AND CONCENTRATES

The average results recorded in Table XI for the total increase in metabolism resulting from the consumption of 1 kg. of dry matter of the several rations—i. e., for the so-called "work of digestion" in the widest sense—are far from being in accord with common conceptions. Unconsciously misled by an unfortunate terminology, we have been accustomed to think of the more coarse and woody feeds, like hay, straw, stover, etc., as requiring a greater expenditure of energy in their digestion and assimilation than the more concentrated and highly digestible grains, for example. It may be somewhat surprising, therefore, to note the relatively small differences found in this respect between different classes of feeding stuffs, as shown by the averages of Table XI and by figure 2. For example, the expenditure of energy caused by maize meal in experiment 179 was almost as great as that caused by

the clover hay with which it was fed, while in experiment 211 it was apparently distinctly greater than that due to the mixed hay consumed with it. Grain mixture No. 1 decidedly exceeded timothy hay in this respect, and grain mixture No. 2 was nearly equal to alfalfa hay.

As a matter of fact, however, these results are in general harmony with those of other investigators, particularly Kellner. The senior writer (2, p. 492) pointed out some 12 years ago that the total expenditure of energy consequent upon feed consumption, as computed from Kellner's published experiments, is strikingly uniform for the several materials experimented upon with the exception of wheat gluten, the average results computed per kilogram of dry matter being quite of the same order as those here reported, viz:

*Average energy expenditure per kilogram of dry matter*

| | Calories. |
|---|---|
| Meadow hay | 1,254 |
| Oat straw | 1,014 |
| Wheat straw | 1,138 |
| Extracted straw | 1,160 |
| Starch: | |
| Kühn's experiments | 1,508 |
| Kellner's experiments— | |
| Moderate rations | 1,248 |
| Heavy rations | 903 |
| Peanut oil | [a] 1,727 |
| Wheat gluten: | |
| Kühn's experiments | 2,558 |
| Kellner's experiments | 2,096 |
| Beet molasses | 988 |

Kellner's later experiments (24, ed. 6, p. 160–168) have not yet been published in full, so that it is not possible to make an exact computation of the energy expenditure. In certain cases, however, the percentages of digestible nutrients are reported. If the corresponding amount of metabolizable energy be computed, using the factors given on page 453, and from this the amount of energy gained by the animal subtracted, the difference will represent approximately the energy spent in digestion, etc. The results of such computations are as follows:

*Energy expenditure per kilogram of dry matter, computed from Kellner's experiments*

| Feeding stuff. | Digestible nutrients. | Computed metabolizable energy. | Gain in energy by animal. | Energy expended in feed consumption. |
|---|---|---|---|---|
| | *Gm.* | *Calories.* | *Calories.* | *Calories.* |
| Cottonseed meal | 647 | 2,588 | 1,869 | 719 |
| Peanut meal | 672 | 2,688 | 1,798 | 890 |
| Palm-nut meal | 624 | 2,496 | 1,739 | 757 |
| Linseed meal | 690 | 2,760 | 1,828 | 932 |
| Barley straw | 464 | 1,624 | 747 | 877 |
| Clover hay | 498 | 1,743 | 811 | 932 |
| "Grass hay" | 528 | 1,848 | 803 | 1,045 |
| Rowen | 487 | 1,705 | 747 | 958 |

The approximate results thus computed for the coarse fodders are comparable in a general way with ours upon similar feeds, although

[a] One very high result was rejected.

somewhat lower than Kellner's direct results just cited. Those on the oil meals appear relatively lower than ours, although even then they are not much lower than those for the coarse feeds, but it may be questioned whether the estimates of the metabolizable energy of these feeds are not too low.

### FACTORS OF INCREASED METABOLISM

Even the very approximate and partial analysis of the total heat production which is attempted in the second part of Tables IX, X, and XI serves to show that the degree of uniformity noted in the preceding paragraph, so far from being surprising, was rather to be expected. The notion of a greater expenditure of energy on coarse feeds is based on the idea that this expenditure is largely for mechanical work. The analysis of the heat production attempted on preceding pages, however, even though only approximate, clearly shows that a considerable portion of the increase in heat production is due to other causes. Roughly, from 9 to 17 per cent of the increase is computed to have had its source in the methane fermentation, while from 3 per cent to as much as 30 or 40 per cent appears to have been due to increased muscular activity while standing. The "remainder" may be regarded as consisting of the mechanical work of digestion plus the stimulus which the feed exerted upon the general metabolism of the animal. How large the latter factor is we have no means of determining, but apparently it is not inconsiderable.

It would seem that the energy expended in peristalsis can not be widely different per kilogram for the different classes of feeding stuffs. On the other hand, the work of mastication and rumination has been shown to be distinctly greater for the coarse feeds. On the basis of Paechtner's (39) and of Dahm's (17) experiments on cattle it may be roughly estimated at 100 Calories per kilogram for hay. Zuntz and Hagemann (52) found the work of masticating oats by the horse to be 28 per cent of that required for hay. On this basis an expenditure by cattle of approximately 28 Calories per kilogram of concentrated feeds, may be estimated. If these amounts are subtracted from those shown in the last column of Table XI, the following approximate figures are obtained per kilogram of dry matter consumed for the work of peristalsis plus the food stimulus to the general metabolism:

| COARSE FEEDS | Calories. | CONCENTRATES | Calories. |
|---|---|---|---|
| Timothy hay | 404 | Maize meal | 878 |
| Clover hay (experiment 179) | 340 | Hominy chop | 1,152 |
| Mixed hay | 685 | Wheat bran | 915 |
| Alfalfa hay | 802 | Grain mixture No. 1 | 803 |
| Maize stover | 775 | Grain mixture No. 2 | 872 |

Whether the expenditure of energy in peristalsis in cattle is as small as it appears from recent investigations to be in man and in the carnivora it is impossible to say, but one can hardly avoid the impression that the considerable differences shown by the foregoing figures, and

especially the generally higher results for the concentrates, indicate that the direct stimulation of metabolism is a large factor.

It appears, then, that while the mechanical work required for the digestion of concentrates is somewhat less than that necessary in case of coarse fodders, this difference is more than compensated for by other factors, so that on the whole fully as great an increase in the heat production is caused by the consumption of the concentrates. As a class, concentrates are superior to coarse fodders, not because their consumption involves a less expenditure of energy, but because they contain more metabolizable energy, so that more remains available for body use after that expenditure has been met.

DIFFERENCES BETWEEN FEEDING STUFFS

But while our results do not show the existence of as great differences between the two great classes of feeding stuffs in their effects on the energy expenditure of the body as seems to have been at times assumed, they nevertheless reveal distinct differences even between feeding stuffs of the same class. Thus, among the hays (if the results of experiment 179 for clover hay are accepted) a regular increase is found in the total energy expenditure from timothy hay with an average of 782 Calories through mixed hay and clover hay up to alfalfa with an average of 1,169 Calories. Apparently the legumes cause a distinctly greater increase in the metabolism than the Poaceae (Gramineae). In the case of red clover, the difference, according to the meager results obtained, appears to result chiefly from a stimulation of the metabolism due to standing. With alfalfa, on the contrary, the increase in the standing metabolism is not materially greater than in the case of timothy hay, while that due to fermentation is somewhat less. The chief difference between the two seems to lie either in their effect upon the work of peristalsis or in the degree to which they stimulate the general metabolism. One can hardly doubt that the latter is the chief cause and is naturally inclined to associate it with the higher percentage of protein in the legumes. That other causes may also be operative, however, is indicated by the result on maize stover, which is nearly as high as in the case of alfalfa and shows a similar distribution among the several factors.

Among the concentrates there may be noted particularly the marked effect of maize in both the two not very satisfactory experiments in noticeably increasing the standing metabolism. This result is of special interest in view of Zuntz and Hagemann's observations (52, p. 259) on the stimulating effect of maize upon the metabolism of the horse, which were also made on the standing animal, although no increase in the minor muscular activity is reported. Grain mixture No. 1, containing 43 per cent of maize meal, likewise showed a similar effect, although with grain mixture No. 2, containing 60 per cent of maize, it was much less marked, possibly on account of the lower content of protein (12.5 as compared with 17.5 per cent). The increases caused by wheat bran

and by hominy chop, on the other hand, appear to have affected chiefly the metabolism of the animal when lying.

### INDIVIDUAL DIFFERENCES

Attention was called on pages 460–461 to the existence of individual differences in the effect of the feed on the ratio of the standing to the lying metabolism. These differences seem in some instances to extend also to other factors of the total heat increment. While the single results are more or less variable, this fact seems to be brought out clearly in the averages. The most striking example is afforded by the animals A and B in experiments 190, 200, and 207, for which the following averages may be computed, showing the heat increments per kilogram of feed to have been distinctly greater with steer B than with steer A. This is, of course, the converse of the conclusion recorded in an earlier publication (10).

*Average heat increments of steers A and B per kilogram of dry matter*

| Feeding stuff. | Animal No. | Total increment. | Distribution. | | | |
|---|---|---|---|---|---|---|
| | | | Standing 12 hours. | Rising and lying down. | Methane fermentation. | Remainder. |
| | | *Calories.* | *Calories.* | *Calories.* | *Calories.* | *Calories.* |
| Timothy hay | A | 717 | 113 | 6 | 139 | 459 |
| | B | 890 | 183 | 4 | 126 | 577 |
| Timothy hay and wheat bran | A | 1,066 | 84 | 0 | 140 | 842 |
| | B | 1,288 | 101 | −1 | 144 | 1,044 |
| Timothy hay and grain mixture No. 1. | A | 1,223 | 296 | 7 | 156 | 764 |
| | B | 1,430 | 367 | 1 | 164 | 898 |

No such distinct differences were observed between the other animals, which, however, were all of similar type. While steers C, E, and F showed an increased effect upon the standing metabolism in the order named the difference in the metabolism of the animals when lying shows on the average an approximately equivalent decrease, so that no material difference in the total effect resulted.

### SUMMARY

Tables X and XI include the results of all of our experiments which have been so far computed as to permit their discussion. In seeking to derive from the recorded results for the increased energy expenditure consequent upon the consumption of certain feeding stuffs general averages which may, with the reservations made on previous pages, afford a basis for estimating the energy values of classes of feeding stuffs and of mixed rations, a certain degree of freedom of choice and the exercise of the judgment of the experimenters seems warranted. Of our results, those on clover hay in experiment 186 appear to us particularly questionable. In one period the animal did not lie down during the entire 48 hours, while in two other periods the time spent in lying was much less than normal. Furthermore, there was a considerable difference

between the observed and the computed heat production in four out of six periods, although it is true that this difference was not relatively greater than in experiment 190. Whether these facts are in any degree responsible for what seem abnormally low results and for the very large proportion of the heat increment apparently due to stimulation of the standing metabolism, it is hard to say, but the results differ so widely from all the others that we feel justified in rejecting them, pending other experiments, particularly since the total increment observed in experiment 179 agrees very well with that computed on page 478 from Kellner's experiments. Experiment 212 fails to show any significant difference between alfalfa hay and alfalfa meal, and the two have been averaged together. In the case of maize meal it is difficult to decide which, if either, of the discordant results is worthy of most credit. The figure of only 393 Calories per kilogram for the increase of the metabolism of the animal when lying, however, seems so low that we are inclined to attach greater weight to the later experiment. For grain mixture No. 2 we have used the results computed from periods 1 and 2 in the belief that the heat production in period 3 was rendered abnormally high by the restlessness of the animals, owing to the small bulk of their ration (compare p. 461), although the difference is scarcely significant.

In the Möckern experiments Kellner's results on heavy rations of starch appear to be abnormal in that the methane production was not increased, while much starch escaped digestion. Kühn's results were obtained on rations of coarse fodder and starch alone with a nutritive ratio of about 1 : 20, or even wider—i. e., under conditions seldom or never realized in practice. Kellner's average for medium rations, therefore, would appear to correspond most nearly to normal conditions. The results on peanut oil were irregular in several respects, but the rejection of the very high result with ox D seems justified. Of the computed results of Kellner's experiments, as given on page 478, those for the oil meals seem unquestionably too low and have been rejected.

On the foregoing assumptions we have formulated the following averages for the total energy expenditure resulting from the consumption of 1 kg. of the dry matter of the feeds named. It may not be superfluous to call attention again to the fact that these figures are simply general averages, derived in some instances from quite discordant single results, and that, as both our own and the Möckern experiments show, they are subject to very considerable variations in individual cases.

*Average energy expenditure per kilogram of dry matter eaten*

| COARSE FODDERS | Calories. | CONCENTRATES | Calories. |
|---|---|---|---|
| Timothy hay | 782 | Maize meal | 1,434 |
| Red clover hay | 962 | Hominy chop | 1,365 |
| Mixed hay | 980 | Wheat bran | 1,177 |
| Alfalfa hay | 1,169 | Grain mixture No. 1 | 1,327 |
| "Grass hay" | 1,045 | Grain mixture No. 2 | 1,[illegible]41 |
| Rowen | 958 | Beet molasses | 988 |
| Meadow hay | 1,254 | Starch | 1,248 |
| Maize stover | 1,065 | Peanut oil | 1,727 |
| Barley straw | 877 | Wheat gluten | 2,294 |
| Oat straw | 1,014 | | |
| Wheat straw | 1,138 | | |
| Extracted straw | 1,160 | | |
| Clover hay | 932 | | |

## III. NET ENERGY VALUES AND THEIR COMPUTATION

The method of estimating the nutritive values of the feeding stuffs consumed by farm animals which has been current for many years may from one point of view be characterized in a broad way as a chemical method. On the basis of the fundamental investigations of Henneberg and Stohmann (21, 22) in the early sixties, it sought to determine the amounts of protein, carbohydrates, and fat contained in feeding stuffs in a digestible form, assuming that the groups thus determined had the same physiological values in the nutrition of herbivora as had the corresponding substances in the food of man and carnivora. It is a well-recognized fact, however, that our information regarding both the qualitative and quantitative composition of feeding stuffs is even yet very meager. Moreover, our knowledge of the physiological functions of their ingredients is even more defective, so that, as Kellner (24, p. 15) points out, the advances in our knowledge of the chemistry of plants have not led to a corresponding increase in our knowledge of their nutritive values and have left the methods for the analysis of feeding stuffs largely untouched.

Kellner appears to have been the first to attempt any practical application of the conception of the feed as a source of energy to the body. In 1880, in his investigations upon the relations between muscular activity and metabolism in the horse (23), he determined the additional amount of work which the animal was able to perform as a result of the addition to his rations of starch and of fat. He expressed his results in terms of the percentage of the energy of the starch or fat which was recovered as useful work and called attention to the desirability of determinations of the heats of combustion of nutrients and feeding stuffs. Sixteen years later, after Rubner (40, 41) had published his fundamental work on the replacement values of nutrients and Zuntz and his associates (30, 54) had begun their investigations on the metabolism of the horse from the standpoint of energy, Kellner was able to return to the subject and undertake those extensive investigations with cattle (cited on previous pages) upon which he based his well-known method of comparing feeding stuffs on the basis of their so-called starch values. These are in reality energy values, and, so far as they are the results of direct determinations, they were obtained by substantially the same general experimental methods used in our own investigations, although direct determinations of the heat production were not included.

### VALUES DIRECTLY DETERMINED

The net energy value of a feeding stuff, as stated in the introductory paragraphs, is the energy which remains after deducting from its total chemical energy the two classes of losses which have been discussed in the first two sections of this article—viz, the losses of chemical energy

in the excreta and the increased heat production consequent upon the consumption of the feed. For example, the alfalfa hay consumed by steer E in experiment 208 contained per kilogram of dry matter 4,408 Calories of chemical energy. From the results reported in Tables III and X its net energy value, computed from the average results of periods 4, 5, and 6, is as follows:

*Net energy value of alfalfa hay per kilogram of dry matter*

| | Calories. | Calories. | Calories. |
|---|---|---|---|
| Total chemical energy | | | 4,408 |
| Losses of chemical energy: | | | |
| In feces | 2,062 | | |
| In urine | 243 | | |
| In methane | 266 | | |
| Total | | 2,571 | |
| Increased heat production | | 1,202 | |
| Total losses | | | 3,773 |
| Net energy value | | | 635 |

Computed in practically this way, by subtracting from the gross energy the average losses of chemical energy recorded in Table IV and the average energy expenditure consequent upon the consumption of the feeding stuff as given on page 482, the average net energy values of the feeding stuffs used in these experiments are as follows:

*Net energy values of feeding stuffs per kilogram of dry matter*

| Feeding stuff. | Gross energy. | Losses of chemical energy. | Energy expended in feed consumption. | Net energy values. |
|---|---|---|---|---|
| | *Calories.* | *Calories.* | *Calories.* | *Calories.* |
| Timothy hay | 4,518 | 2,664 | 782 | 1,072 |
| Red clover hay | 4,462 | 2,461 | 962 | 1,039 |
| Mixed hay | 4,393 | 2,479 | 980 | 934 |
| Alfalfa hay [a] | 4,372 | 2,451 | 1,169 | 752 |
| Maize stover | 4,332 | 2,380 | 1,065 | 887 |
| Maize meal | 4,442 | 1,115 | 1,434 | 1,893 |
| Wheat bran | 4,532 | 2,021 | 1,177 | 1,334 |
| Grain mixture No. 1 | 4,685 | 1,621 | 1,327 | 1,737 |
| Grain mixture No. 2 | 4,609 | 1,620 | 1,141 | 1,848 |
| Hominy chop | 4,709 | 1,187 | 1,365 | 2,157 |

[a] Includes alfalfa meal.

Kellner's results when put into the same form are as follows:

*Net energy values of feeding stuffs per kilogram of dry matter: Kellner's results*

| Feeding stuff. | Gross energy. | Losses of chemical energy. | Energy expended in feed consumption. | Net energy values. |
|---|---|---|---|---|
| | *Calories.* | *Calories.* | *Calories.* | *Calories.* |
| Meadow hay | 4, 433 | 2, 260 | 1, 254 | 919 |
| Oat straw | 4, 436 | 2, 848 | 1, 014 | 574 |
| Wheat straw | 4, 444 | 3, 062 | 1, 138 | 244 |
| Extracted straw | 4, 147 | 1, 013 | 1, 160 | 1, 974 |
| "Grass hay" [a] | | | 1, 045 | 803 |
| Rowen [a] | | | 958 | 747 |
| Barley straw [a] | | | 877 | 747 |
| Clover hay [a] | | | 932 | 811 |
| Starch | 4, 152 | 1, 101 | 1, 248 | 1, 803 |
| Peanut oil | 9, 457 | 4, 165 | 1, 727 | 3, 565 |
| Wheat gluten | 5, 579 | 1, 974 | 2, 096 | 1, 509 |
| Beet molasses | 3, 743 | 945 | 988 | 1, 810 |

[a] As estimated on page 478.

Very striking is the relatively low value for alfalfa hay, due in part to somewhat large losses in the excreta but chiefly to its marked effect in stimulating the metabolism. It is needless to add that this loss does not affect its special value as a source of protein, but as a source of energy it appears to have been distinctly inferior to timothy hay or even to maize stover.

## APPLICATION OF RESULTS TO OTHER FEEDING STUFFS

It is obviously impracticable to apply the laborious methods of respiration and calorimeter experiments to all the vast number of feeding stuffs now in use. It is necessary to select a few typical representatives of different groups and to endeavor to apply the results obtained as well as possible to other similar materials. This Kellner sought to do in his later and as yet unpublished experiments. In the practical application of his results, however, Kellner failed to free himself from the older point of view. Aside from what seems to us the unfortunate and unnecessary concession to established usage involved in expressing energy values in terms of matter, he approached the whole problem, as was quite natural, along the lines of the prevailing chemical methods. Determining first the net energy values of the simple nutrients, he applied these values to the digestible nutrients of feeding stuffs and found that in most cases the resulting energy values were materially higher than those obtained by direct experiments on animals. In the case of coarse fodders this deficit in the observed energy values was found to be approximately proportional to the total content of crude fiber, and by subtracting from the computed energy value 1.36 Calories per gram of total crude fiber results were obtained corresponding fairly well to those directly observed.

For finer materials like chaff, presumably requiring a less expenditure for mastication, 0.70 Calorie per gram of total crude fiber is deducted. For green forage containing 16 per cent or more of crude fiber the same deduction is made as for dry forage and for that containing 4 per cent or less of crude fiber, the same as for chaff, while between these limits a sliding scale is used (24, 1905, p. 593-594). For concentrates a factor (Wertigkeit) is estimated from the direct results on similar feeds by which the energy value computed from the digestible nutrients is multiplied to obtain the actual value.

The method of computation just outlined is not only somewhat complicated but is essentially based on the older view which regarded the feed in the light of a source of matter to the body. The digestible protein, carbohydrates, and fat are still the basis of the calculation, although certain more or less empirical corrections are applied to their computed effects. The energy content of a feeding stuff, however, is just as definite a quantity as its content of protein, carbohydrates, or fats, and it is entirely possible to trace the distribution of that energy in the body quite independently of any knowledge of the chemical composition of the materials. Not only so, but we believe that in discussing energy values there are distinct advantages as regards simplicity, and perhaps also as regards accuracy, in cutting loose entirely from the conventional data regarding chemical composition and digestion coefficients, as has been done in reporting our experiments on preceding pages, and in dealing directly with quantities of energy.

In making this statement we would by no means be understood to stigmatize comparisons based on chemical methods as either valueless or superfluous. The problems of nutrition are too complex and too difficult for us to refuse any light that can be thrown on them by any method, and the energy relations touch only one phase of them. The point is that in whatever degree their energetic aspects can be separated from their chemical aspects, to that extent we possess two independent methods of approach to them.

## COMPUTATION OF NET ENERGY VALUES

The computation from the results of metabolism experiments or from the data of ordinary feeding tables in the manner just indicated of the net energy value of a feeding stuff which has not been the subject of direct experimental investigation with the respiration apparatus or calorimeter may be made a comparatively simple matter. The net energy value is equal to the metabolizable energy minus the energy lost as heat. It was shown on pages 450-451 that the metabolizable energy may be determined experimentally without special difficulty and with a good degree of accuracy by means of the ordinary metabolism experiment in which the energy of the feed, feces, and urine is directly determined and that

of the methane estimated from the amount of carbohydrates digested. When this is not practicable, it was further shown that the metabolizable energy may be estimated from the total digestible organic matter by the use of the factors given on pages 451–453. In one or other of these ways it is not difficult to compute approximately the metabolizable energy of the more common feeding stuffs, while the subtraction from this of the average energy expenditure due to feed consumption will give the net energy value. To illustrate, E. W. Allen,[1] gives the following data for average alfalfa hay, oat straw, and wheat bran:

*Percentage of dry matter and digestible food ingredients of feeding stuffs*

| | Alfalfa hay. | Oat straw. | Wheat bran. |
|---|---|---|---|
| Total dry matter | 91. 6 | 90. 8 | 88. 5 |
| Digestible: | | | |
| Protein | 10. 58 | 1. 20 | 12. 01 |
| Carbohydrates | 37. 33 | 38. 64 | 41. 23 |
| Fats | 1. 38 | 0. 76 | 2. 87 |
| Total digestible | 49. 29 | 40. 60 | 56. 11 |

The sum of the digestible protein, carbohydrates, and fat equals, of course, the total digestible organic matter, irrespective of its chemical composition. Each gram of digestible organic matter, according to the averages on pages 451–453, would contain 3.5 Calories of metabolizable energy in the coarse fodders and 3.9 Calories in the bran. The average losses of energy in heat production per kilogram of feed would be the amounts shown on page 482 reduced to the average water content of the feed, as follows:

| | |
|---|---|
| Alfalfa hay | 1,169×0.916=1,071 Calories. |
| Oat straw | 1,014×0.908= 921 Calories. |
| Wheat bran | 1,138×0.885=1,007 Calories. |

The computation of the net energy values is therefore as follows:

Alfalfa hay (3.5 Calories×492.9)−1,071 Calories=654 Calories per kilogram=29.7 T. per 100 pounds.
Oat straw (3.5 Calories×406.0)−921 Calories=500 Calories per kilogram=22.7 T. per 100 pounds.
Wheat bran (3.9 Calories×561.1)−1,007 Calories=1,181 Calories per kilogram=53.6 T. per 100 pounds.

The methods of computation just illustrated are perhaps open to the charge of being to a degree summary and empirical. The idea of basing such computations on the energy values of the single ingredients may be fundamentally more scientific, but unfortunately at present it is an impracticable ideal on account of our deficient knowledge of the chemistry of feeding stuffs and of the physiological values of their ingredients. While investigation along both these lines is highly important and desirable, yet for a long time to come the data on which to base the practice of stock feeding will have to be obtained by more direct even

[1] Allen, E. W., The feeding of farm animals. U. S. Dept. Agr. Farmers' Bul. 22 (rev.), p. 8–9. 1901.

if less fundamental methods. Kellner's scheme recognizes this fact and his deduction for crude fiber and his factors for relative values (Wertigkeit) are at bottom simply a method of applying the aggregate net results on typical feeding stuffs to other materials. The method here proposed seeks to do exactly the same thing more directly and simply, relating the energy content and the necessary deductions to the total dry matter or total digestible matter of the feeding stuff, independently of its chemical composition. It is true that the data for so doing are somewhat meager, but, except as Kellner has utilized unpublished data in the formulation of his tables, they are just as abundant in the one case as in the other. It is greatly to be regretted that Kellner's results have not yet been published in full. When they become available they will doubtless greatly broaden the basis for such computations.

## SUMMARY

There are reported the results of 76 experiments with the respiration calorimeter upon nine steers in which the balance of matter and of energy was determined.

The losses of feed energy from the animal are of two classes: (1) Losses of unused chemical energy in the feces, urine, and methane; and (2) losses in the form of heat due to the increased metabolism consequent upon the ingestion of feed.

(1) LOSSES OF CHEMICAL ENERGY.—The losses of energy in methane and urine were relatively greater on light than on moderately heavy rations.

Neither the losses of energy in the feces nor the total losses showed a distinct relation to the amount of feed consumed.

Individual differences between animals had no very material influence on the losses of chemical energy.

The losses of energy in methane may be computed approximately from the amount of total carbohydrates digested.

The metabolizable energy per kilogram of digested organic matter showed but slight variations within the same class of feeding stuffs.

(2) LOSSES OF HEAT CONSEQUENT UPON FEED CONSUMPTION.—The heat production is notably greater during standing than during lying, and the difference is greater on heavy than on light rations.

The increment of heat production during standing is affected by the individuality of the animal and by the kind of feed consumed.

An approximate partial analysis of the heat production of the animal into its principal factors is attempted.

The average energy expenditure consequent upon the consumption of 1 kg. of dry matter is reported for 11 different feeding stuffs.

The expenditure of energy arising from the consumption of the coarse feeds is not on the whole materially greater than in the case of the concentrates.

The increased muscular work of the digestive organs appears to be a relatively small factor of the increased heat production.

A scrub steer showed a somewhat greater increment of metabolism consequent upon feed consumption than did a pure-bred beef animal.

(3) NET ENERGY VALUES.—A summary of the average net energy values obtained in these experiments for 11 different feeding stuffs is given.

A simple method is outlined for computing net energy values, in the absence of direct determinations, from metabolism experiments or from the data of ordinary feeding tables.

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# AIR AND WIND DISSEMINATION OF ASCOPORES OF THE CHESTNUT-BLIGHT FUNGUS

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## HISTORICAL INTRODUCTION

Wind dissemination of the chestnut-blight fungus (*Endothia parasitica* (Murr.) And.) was first suggested by Murrill (13)[2] in 1906, although he apparently had only the pycnospores in mind, as is shown by the following quotation:

> Later the fruiting pustules push up through the lenticels and give the bark a rough, warty appearance; and from these numerous yellowish-brown pustules millions of minute summer spores emerge from day to day in elongated reddish-brown masses to be disseminated by the wind and other agencies, such as insects, birds, squirrels, etc.

A few years later, in a discussion of the means of spreading the disease, Hodson (9) says:

> Wind is probably the principal agency, but the spores are no doubt carried by animals, birds, insects, and by shipment of infected material.

He also cited some observations to substantiate the wind-dissemination theory, but it was not brought out clearly whether he had in mind the ascopores or the pycnospores only. A similar opinion is expressed by Mickleborough (12) a little later. After speaking of both the ascopores and the conidal, or summer, spores, he states:

> The minute spores are carried by the wind, on the feathers of birds, and the fur of squirrels.

Referring to the spore horns, Mickleborough writes:

> These threads are dissolved and washed away by the rain and the spores are blown about by the wind.

There are two possible ways in which pycnospores might be disseminated by the wind: First, by the direct transport of spore horns or small fragments of these structures; second, by the transport of dust particles bearing spores previously washed down by rains.

Fulton (4) reports experiments which indicate that the former method of transport of pycnospores is of little importance in the spread of the disease. He concludes his discussion of this topic with the following statement:

> It seems likely the detachment was largely of small bits of the tendrils made up of large numbers of spores, and that these are too heavy to be carried great distances;

[1] The writers received valuable assistance in this work from Mr. R. C. Walton, also an agent, Investigations in Forest Pathology.

[2] Reference is made by number to "Literature cited," p. 525-520.

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and suggests that under natural conditions infection may be spread short distances by the wind.

The second possibility is brought out by Metcalf and Collins (11), as may be noted in the following quotation:

> As both kinds of spores appear to be sticky, there is no evidence that they are transmitted by wind except where they may be washed down into the dust and so blown about with the dust.

While it has not yet been demonstrated that pycnospores are carried in this way, the tests of Heald and Gardner (7) on the longevity of pycnospores in soil give added plausibility to the theory, since these spores were found to persist in the soil between periods of rain and were able to withstand complete desiccation in the laboratory for months.

Attention was first directed to the strong probability of wind dissemination of ascopores by Rankin (14), who reported their forcible expulsion. In a later report the same writer (15) makes the following statement:

> Under moist conditions the ascospores are shot forcibly out in the air where they can be caught up by the wind and carried for a considerable distance. The speaker found ascopores being shot from mature pustules during every rainy period last summer. * * * The question at once arises, Why could not these ascospores once shot into the air be carried long distances and, owing to their abundance, cause a large majority of the infection?

After carrying out field experiments during the summer of 1912, Rankin (16), referring to ascospores, says:

> They are shot out in vast numbers with every rain during the summer and are carried by the wind.

Detailed field work on dissemination was carried out by Anderson (1) and his assistants for the Pennsylvania Chestnut Tree Blight Commission (2). These publications confirm the statement of Rankin that expulsion of spores takes place only when the pustules are moist. The seasonal duration of shooting under natural conditions was not determined, as the field tests were confined to the month of August. Under artificial conditions in the laboratory, the time required for moistened bark bearing perithecia to begin the expulsion of spores was determined, the shortest time recorded being three minutes.

The duration of the shooting period following a rain was determined by artificial tests in either the field or laboratory, performed by soaking the specimens or drenching cankers with water. The maximum duration recorded was five hours and two miniutes. While these tests under artificial conditions gave suggestive results, they were not necessarily a reliable indication of what would happen under natural conditions.

It was also determined that bark kept constantly moistened continued to expel spores for a maximum period of 25 days, and the point was emphasized that no continuous rainy weather would be longer. The fact that ascospores expelled during a rain would be washed down to

the ground without being carried any appreciable distance is not mentioned. Since they germinate at once in rain water, the great bulk of such spores would be lost for anything but very local infections. The really important point would appear to be the length of time shooting continues after a rain ceases, for at that time the conditions of the atmosphere would be such as to favor a wider dissemination. This question does not seem to have been satisfactorily answered. The data given on height and horizontal distance of projection, as well as the rate of expulsion, certainly indicate the importance of wind transport of spores following rainy periods.

The spore content of the air was studied by means of aspirator tests and exposure plates. In this work, carried out during dry weather, Anderson and his assistants failed to get positive results under natural conditions in the field. They report the use of over 100 exposure plates and tests of 500 liters of air without finding a single spore of the chestnut-blight fungus. Tests made of aspirated air and by exposure plates gave positive results, however, when the cankers were artificially drenched with water. For the aspirator tests the horizontal distances of the aspirator opening from the canker varied from 2 inches to 5 feet (?) and the maximum vertical distance was 22 feet.

The tests made by exposing agar plates under artificial conditions in the field again pointed to the probability of wind dissemination, but one is forced to admit that they were not conclusive, since the conditions were so different from the natural in that the cankers were drenched with water artificially instead of waiting for a rain. The results with exposure plates may be summed up as follows: No spores of the chestnut-blight fungus were obtained under natural conditions in the field during dry weather; by the use of artificially drenched cankers spores were obtained at distances varying from 1 inch to 51 feet, with very few at the maximum distance.

The final and most conclusive argument in favor of wind dissemination in the minds of the authors cited was afforded by inoculations made by offering an opportunity for wind-borne spores to be introduced into wounds. There is little doubt in the minds of the writers of this paper that infection did take place in the way claimed, but it should be pointed out that a covering of cotton would not prevent spores from being washed into the wounds by rains (6). A fairly compact mass of cotton has been shown to retain but few of the pycnospores present in water passing through it. It must therefore be admitted that, under the conditions of the experiments reported, infection by spores washed down by rains was one of the possibilities.

It is interesting to note in this connection that Kittredge (10), as a result of field observations on the spread of the disease around a center of infection, arrives at the following conclusion:

> The location of infected trees in partially infected groups of sprouts shows that wind is not the prime factor in the distribution of the spores.

The author admitted, however, that the observations reported were rather meager in support of this conclusion.

## PURPOSE AND SCOPE OF PRESENT WORK

Since most of the previous work on wind dissemination of the chestnut-blight fungus which yielded positive results was done under artificial conditions, it was the aim of the present writers to study the problem under absolutely natural conditions. Briefly stated, the purpose of these tests was to determine whether or not, and if so, to what extent, wind [1] acts as an agent in dissemination of the spores of this fungus. It was also the object of the work herein recorded to ascertain at what particular times under natural conditions spores of *Endothia parasitica* are prevalent in the air, the possible distances transported by the wind, and the kind of spores (whether ascospores or pycnospores).

The locality chosen in which to conduct our tests was a 4-acre plot of native chestnut (*Castanea dentata*) coppice near West Chester, Pa. The trees in this plot ranged from 4 to 8 inches in diameter and all were badly infected with the chestnut blight, many having already succumbed.

In these tests, which covered a period of 36 consecutive days during August and September, 1913, four methods were employed in studying the points in question. To determine the prevalence of spores of *Endothia parasitica* in the air at particular times and places a series of 756 exposure plates was made. The occurrence of ascospore expulsion was detected and its exact period of duration ascertained by the examination of ascospore traps in the shape of object slides supported over perithecial pustules on the trees. The number of spores present in the air was determined quantitatively by the aspirator method. Rather prolonged exposures of water spore traps, consisting of sterile water in dishes, were made to secure additional information as to the kind of spores in the air, the periods of occurrence, and the distance transported.

## EXPOSURE-PLATE TESTS

In testing the spore content of the air among diseased trees in the field for the presence of spores of *Endothia parasitica* the exposure of sterile poured plates of chestnut-bark agar proved to be the most satisfactory method. The use of chestnut-bark agar [2] was found advantageous, since this medium inhibits the development of bacterial colonies and retards the growth of rapid-growing fungi, spores of which are

[1] Falck has pointed out the importance of convection currents in the dissemination of ascospores. (Falck, Richard. Über die Luftinfektion des Mutterkornes (Claviceps purpurea Tul.) und die Verbreitung pflanzlicher Infektionskrankheiten durch Temperaturströmungen. *In* Ztschr. Forst- u. Jagdw., Jahrg. 43, No. 3, p. 202-227, 4 fig., 1911.) For this reason we have used the word "air" in the title of the present paper.

[2] Chestnut-bark agar was made according to the following formula: Add 50 gm. of finely chopped or ground air-dry chestnut bark to 1,000 c. c. of distilled water and boil for 15 minutes. Filter through cheesecloth or absorbent cotton and add water to make up to 1,000 c. c. Add 15 gm. of agar and boil until the agar has melted; then cool to 60° C. or under, clear with the whites of two eggs, filter, and sterilize in the autoclave.

present in the air. At the same time the growth of *E. parasitica* on this medium is vigorous and characteristic.

As supports or stations on which to expose the plates, it was found convenient and satisfactory to make use of the numerous large flat-topped stumps scattered throughout the coppice stand of diseased trees. To facilitate the recording of data, all of the stumps used were numbered with crayon and carefully described and located with regard to surrounding trees (fig. 1). Here it may be mentioned, however, that other supports, such as the top rail of a fence or the top of a stake driven into the ground, were used in case of emergency attendant upon certain weather conditions.

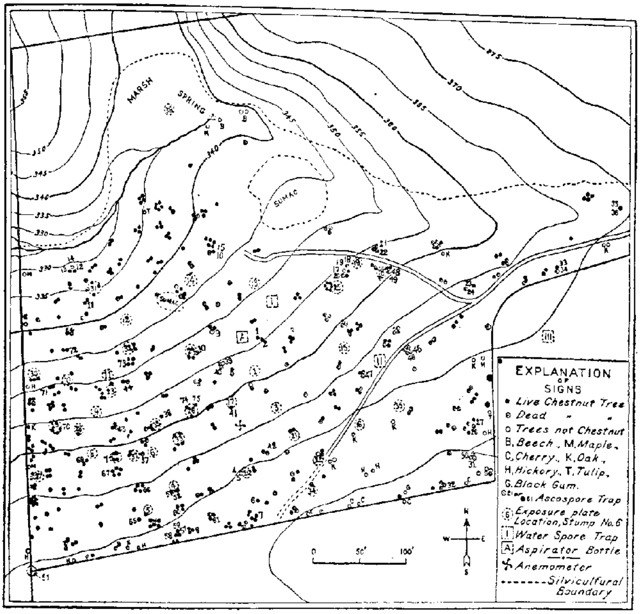


FIG. 1.—Map of chestnut coppice growth at West Chester, Pa., in and near which the experiments on wind dissemination of the chestnut-blight fungus were carried out.

The stumps, rails, and stakes used for this purpose were all of such an age or nature that they were entirely free from lesions of the chestnut blight.

Under conditions of ordinary fair weather the routine followed in making the exposures was similar throughout the tests. Plates were exposed at the rate of one about every half hour during the day, and the average length of exposure was about 5 minutes for each plate during the first 18 days. Then it was found advisable to lengthen the time of exposure, and thereafter 10 minutes, more or less, was the usual time allowed. Wind direction determined what stations were utilized each day, since an

effort was usually made to expose plates at stations where there were many diseased trees to the windward.

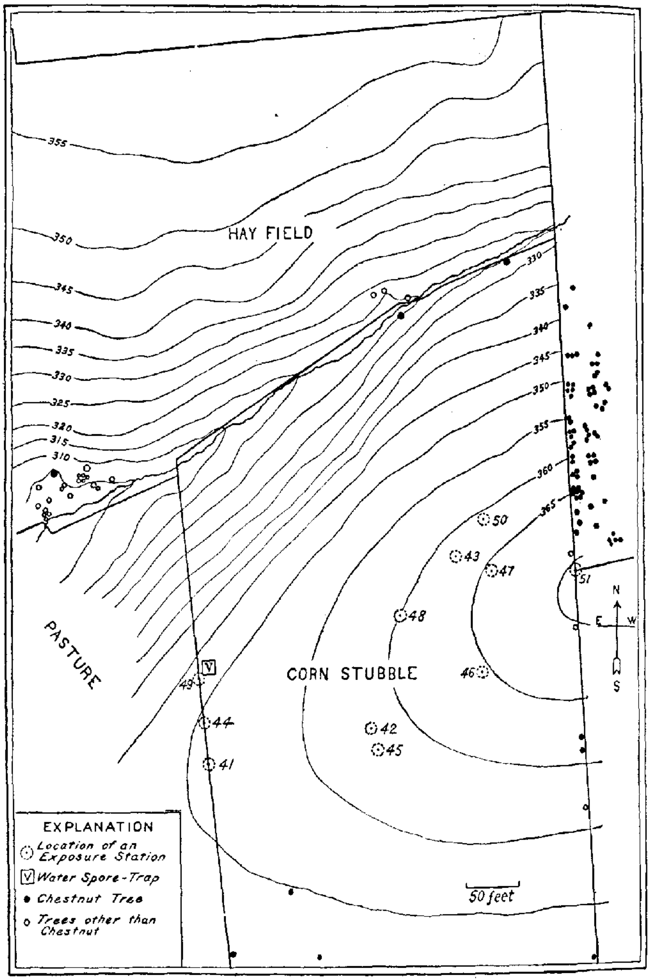


FIG. 2.—Map showing the location of some of the important outlying exposure-plate stations. Station 51 is at the corner of the plot represented in figure 1.

During wet weather the routine was often varied considerably, especially just after the cessation of a rain. At such times plates were often exposed in more rapid succession, even to the extent of exposing several in different locations at about the same time.

An anemometer was erected in the experimental plot, and from the successive readings of this instrument the wind velocities were computed. Continuous records of temperature were secured by means of a thermograph located in a standard instrument shelter near the plot, and by use of a rain gauge the exact rainfall in inches was determined. As complete data as possible were also secured relative to the exact duration of all rains.

In describing and locating the stations used for exposure plates, measurements were made to the nearest diseased trees and to the nearest lesions, the horizontal distance being recorded. To supplement the description, detailed topographic maps were made, showing the location of each station (figs. 1 and 2).

The exposed plates were incubated at room temperature, and two records were usually taken. First, at the end of three days after exposure all fungous and bacterial colonies visible were marked and counted, and those suspected of being *Endothia parasitica* were especially noted. After six or seven days of incubation the final record on each plate was taken. This included the total number of fungi, the number of bacterial and yeast colonies, and the number of colonies of *E. parasitica*, if any were present. In case of doubt as to the identity of the latter, owing to crowding by other colonies, transfers were made to 3 per cent dextrose agar, on which medium the growth of this fungus is even more characteristic than on chestnut-bark agar.

The results obtained in the exposure-plate tests are presented in a somewhat summarized form in Tables I and II.

TABLE I.—*Summary of exposure-plate tests at West Chester, Pa., in 1913, giving number of fungous colonies caught*

| Date of cultures. | Number of plates exposed. | Length of exposure for each plate. | Total time represented. | Rainfall. | Number of fungous colonies in any plate. Maximum. | Minimum. | Total number of fungous colonies. | Total number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| | | *Minutes.* | *H. m.* | *Inches.* | | | | |
| Aug. 19 | 18 | 5 to 6 | 1 30 | 0.40 | 81 | 3 | 332 | 1 |
| 20 | 22 | 6 to 10 | 2 10¼ | 0 | 24 | 1 | 227 | 0 |
| 21 | 19 | 5 to 7 | 1 48½ | 0 | 20 | 0 | 168 | 0 |
| 22 | 20 | 1½ to 7½ | 1 44 | 0.25 (22–23) | 35 (22–23) | 2 | 340 | 0 |
| 23 | 25 | 4 to 8 | 2 10 | | 28 | 1 | 250 | 0 |
| 24 | 17 | 4½ to 6 | 1 28¼ | 0 | 47 | 1 | 206 | 0 |
| 25 | 19 | 4 to 6 | 1 34 | 0 | 75 | 5 | 419 | 0 |
| 26 | 20 | 4 to 7 | 1 48½ | 0 | 95 | 8 | 374 | 0 |
| 27 | 28 | 4 to 9½ | 2 44½ | 0.175 | 70 | 4 | 440 | 04 |
| 28 | 18 | 4 to 6½ | 1 32 | 0 | 20 | 2 | 190 | 0 |
| 29 | 19 | 4 to 5½ | 1 30½ | 1.10 (29–30) | 51 (29–30) | 1 | 309 | 0 |
| 30 | 22 | 5 to 6½ | 1 57¼ | | 146 | 0 | 303 | 0 |
| 31 | 20 | 4 to 7 | 1 48 | 0 | 32 | 1 | 201 | 1 |
| Sept. 1 | 19 | 5 to 6½ | 1 44 | 0 | 12 | 1 | 122 | 0 |
| 2 | 19 | 4 to 6 | 1 37 | 0 | 30 | 3 | 213 | 0 |
| 3 | 19 | 5 to 6½ | 1 37½ | 0 | 10 | 1 | 68 | 2 |
| 4 | 18 | 5 | 1 30 | 0 | 48 | 5 | 245 | 1 |
| 5 | 18 | 5 to 6 | 1 31 | 0 | 40 | 0 | 179 | 1 |

TABLE I.—*Summary of exposure-plate tests at West Chester, Pa., in 1913, giving number of fungous colonies caught*—Continued

| Date of cultures. | Number of plates exposed. | Length of exposure for each plate. | Total time represented. | Rainfall. | Number of fungous colonies in any plate. Maximum. | Minimum. | Total number of fungous colonies. | Total number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| | | *Minutes.* | *H. m.* | *Inches.* | | | | |
| Sept. 6.. | 18 | 5 to 10½ | 2 14¾ | 0 | 6 | 0 | 39 | 0 |
| 7.. | 19 | 7 to 11¾ | 3 4¾ | 0.37 | 22 | 1 | 186 | 0 |
| 8.. | 27 | 7¼ to 12 | 4 16 | | 15 | 1 | 195 | 9 |
| 9.. | 18 | 6 to 11½ | 2 23 | 0 | 31 | 2 | 194 | 2 |
| 10.. | 18 | 8 to 11¾ | 2 50¼ | 0 | 31 | 0 | 138 | 0 |
| 11.. | 18 | 9½ to 14¼ | 3 19¼ | 0 | 25 | 0 | 118 | 0 |
| 12.. | 18 | 5 to 14 | 3 12⅓ | 0.095 | 41 | 1 | 121 | 0 |
| 13.. | 19 | 7 to 13 | 3 5¼ | | 73 | 2 | 332 | 0 |
| 14.. | 18 | 9 to 11¼ | 3 9½ | 0 | 20 | 2 | 126 | 0 |
| 15.. | 18 | 9½ to 11 | 3 6 | 0 | 20 | 6 | 202 | 0 |
| 16.. | 19 | 9½ to 27 | 3 34½ | 0 | 28 | 0 | 251 | 0 |
| 17.. | 17 | 9½ to 15½ | 3 13¾ | | 36 | | | |
| | | | | 0.26 | | 2 | 221 | [a]2 |
| 18.. | 28 | 9¾ to 13½ | 5 15¼ | | 67 | | | |
| | | | | 0.68 | | 1 | 539 | 55 |
| 19.. | 26 | 10¼ to 17 | 5 24½ | | 400 | | | |
| | | | | 0.09 | | 1 | 814 | 7 |
| 20.. | 28 | 9¼ to 18 | 6 13¾ | | 23 | | | |
| | | | | 0.43 | | 1 | 194 | 90 |
| 21.. | 25 | 3½ to 20 | 5 52½ | 0.10 | 160 | 5 | 1,187 | 160 |
| | | | | 0.73 | | | | |
| 22.. | 36 | 6½ to 30 | 9 50 | | 60 | 0 | 494 | 2 |
| 23.. | 24 | 12¼ to 23 | 6 36 | 0 | 30 | 0 | 261 | 0 |

[a] From wind-blown bark fragments.

TABLE II.—*Detailed record of all exposure plates in which spores of Endothia parasitica were caught at West Chester, Pa., in 1913*

| Plate No. | Date of exposure. | Rainfall. | Time elapsed since cessation of rain. D. | h. | m. | Length of exposure. | Horizontal distance to nearest blight lesion. | Number of spores of Endothia parasitica caught. |
|---|---|---|---|---|---|---|---|---|
| | | *Inches.* | | | | *Minutes.* | *Feet.* | |
| 4008 | Aug. 19 | 0.4 | | 6+ | 0 | 5 | [a]1½ | 1 |
| 4376 | Aug. 27 | .12 | | | 42 | 9⅓ | 4½ | 1 |
| 4382 | ....do | .12 | | 1 | 44 | 6 | 15½ | 3 |
| 4383 | ....do | .055 | | | 18 | 6½ | 15½ | 16 |
| 4384 | ....do | .055 | | | 20 | 6 | 11 | 21 |
| 4385 | ....do | .055 | | | 22 | 8 | 25 | 33 |
| 4386 | ....do | .055 | | | 33 | 5 | 4½ | 20 |
| 4460 | Aug. 31 | 1.10 | 1 | 10+ | 0 | 5 | [a]2 | 1 |
| 4509 | Sept. 3 | 1.10 | 5 | 0 | 0 | 5 | [a]2 | 2 |
| 4529 | Sept. 4 | 1.10 | 5 | 0 | 0 | 5 | [a]2 | 1 |
| 4553 | Sept. 5 | 1.10 | 6 | 0 | 0 | 5 | [a]2 | 1 |
| 4599 | Sept. 8 | .37 | | 5+ | 0 | 10½ | 11 | 2 |
| 4600 | ....do | .37 | | 5 | 1 | 11½ | 14 | 1 |
| 4602 | ....do | .37 | | 5 | 21 | 12 | [a]1 | 1 |
| 4606 | ....do | .37 | | 7 | 0 | 9¼ | [a]2 | 1 |
| 4609 | ....do | .37 | | 8 | 30 | 12 | [a]1½ | 1 |
| 4610 | ....do | .37 | | 9 | 0 | 10 | [a]2½ | 1 |
| 4617 | ....do | .37 | | 13 | 0 | 8 | [a]2 | 1 |
| 4619 | ....do | .37 | | 14 | 0 | 9 | [a]2 | 1 |
| 4626 | Sept. 9 | .37 | 1 | 8 | 0 | 6 | [a]2 | 1 |
| 4627 | ....do | .37 | 1 | 8 | 30 | 8½ | [a]4½ | 1 |

[a] Stumps more or less overhung by diseased sprouts.

TABLE II.—*Detailed record of all exposure plates in which spores of Endothia parasitica were caught at West Chester, Pa., in 1913*—Continued

| Plate No. | Date of exposure. | Rainfall. | Time elapsed since cessation of rain. | | | Length of exposure. | Horizontal distance to nearest blight lesion. | Number of spores of Endothia parasitica caught. |
|---|---|---|---|---|---|---|---|---|
| | | *Inches.* | *D.* | *h.* | *m.* | *Minutes.* | *Feet.* | |
| 4772 | Sept. 17 | 0.095 | 4 | 0 | 0 | 10 | [a] 2½ | 1 |
| 4784 | .....do | .095 | 4 | 0 | 0 | 10½ | [a] 2 | [b] 1 |
| 4787 | Sept. 18 | .26 | | 1 | 51 | 9¾ | 15½ | 11 |
| 4788 | .....do | .26 | | 1 | 54 | 10 | 16 | 16 |
| 4789 | .....do | .26 | | 2 | 0 | 10 | 4½ | 9 |
| 4790 | .....do | .26 | | 2 | 19 | 13 | 11 | 4 |
| 4791 | .....do | .26 | | 2 | 35 | 11 | 7 | 5 |
| 4792 | .....do | .26 | | 2 | 48 | 11 | 15½ | 2 |
| 4793 | .....do | .26 | | 2 | 54 | 12 | [a] 4½ | 2 |
| 4795 | .....do | .26 | | 3 | 25 | 11 | 15½ | 2 |
| 4796 | .....do | .26 | | 3 | 34 | 11 | 17½ | 1 |
| 4797 | .....do | .26 | | 3 | 58 | 10¼ | 16 | 1 |
| 5006 | .....do | .26 | | 9 | 14 | 13½ | [a] 1 | 1 |
| 5010 | .....do | .26 | | 11 | 19 | 11 | [a] 2 | 1 |
| 5018 | Sept. 19 | .68 | | 1 | 23 | 11¼ | 4½ | 2 |
| 5020 | .....do | .68 | | 2 | 7 | 12½ | 27 | 1 |
| 5027 | .....do | .68 | | 6 | 4 | 11½ | [a] 2 | 1 |
| 5029 | .....do | .68 | | 7 | 8 | 12¾ | [a] 5 | 1 |
| 5033 | .....do | .68 | | 10 | 5 | 10¼ | [a] 5 | 1 |
| 5037 | .....do | .68 | | 12 | 7 | 13½ | 27 | 1 |
| 5041 | Sept. 20 | .09 | | 1 | 55 | 16 | 217 | 10 |
| 5042 | .....do | .09 | | 2 | 0 | 13 | 195 | 7 |
| 5043 | .....do | .09 | | 2 | 1 | 14 | 110 | 4 |
| 5044 | .....do | .09 | | 2 | 8 | 12¾ | 27 | 22 |
| 5045 | .....do | .09 | | 2 | 19 | 12¼ | 85 | 11 |
| 5046 | .....do | .09 | | 2 | 23 | 15 | 180 | 7 |
| 5047 | .....do | .09 | | 2 | 25 | 15 | 237 | 10 |
| 5048 | .....do | .09 | | 2 | 45 | 13½ | 7 | 6 |
| 5049 | .....do | .09 | | 3 | 12 | 11½ | 11 | 7 |
| 5050 | .....do | .09 | | 3 | 37 | 13 | 11 | 3 |
| 5051 | .....do | .09 | | 3 | 45 | 16¾ | 86 | 1 |
| 5052 | .....do | .09 | | 4 | 29 | 10 | 4½ | 1 |
| 5053 | .....do | .09 | | 5 | 14 | 13½ | 5 | 1 |
| 5069 | Sept. 21 | [c] .35 | | | 0 | 14½ | 19 | 12 |
| 5070 | .....do | .35 | | | 5 | 10½ | 17½ | 20 |
| 5071 | .....do | .08 | | | 15 | 20 | 17½ | 62 |
| 5072 | .....do | .08 | | | 17 | 19 | 19 | 24 |
| 5073 | .....do | .08 | | | 44 | 13½ | 17½ | 19 |
| 5074 | .....do | .08 | | | 56 | 15¼ | 14 | 1 |
| 5075 | .....do | .08 | | 1 | 1 | 12 | 7 | 2 |
| 5087 | .....do | [c] .07 | | | 20 | 14½ | 19 | 20 |
| 5098 | Sept. 22 | .73 | | 6 | 0 | 16 | 77 | 1 |
| 5102 | .....do | .73 | | 6 | 30 | 15 | 1½ | 1 |

[a] Stumps more or less overhung by diseased sprouts. [b] From fragments of bark. [c] Approximately.

To supplement the tables, it may be well to give in chronological order a more detailed record of the actual routine pursued.

A rain occurred the night previous to August 19, and examination of the ascospore traps (see "Ascospore-trap tests") showed that abundant expulsion of ascospores had occurred. But when the first plates were exposed too long a time had evidently intervened since the rain, as no positive results were obtained, except that one colony of *Endothia parasitica* developed in a plate exposed in the early afternoon.

The next two days were fair, and as was expected for these weather conditions, no spores of this Endothia were caught. In the evening of August 22 there was a rain of 0.25 inch; 6 plates, therefore, were exposed early the next morning before the sun had dried the vegetation. Although the ascospore traps gave evidence that expulsion had occurred, no positive results were obtained, which is explained by the fact that again too long a time had elapsed after the rain ceased.

Dry, hot weather now continued until the afternoon of August 27, and the exposure plates yielded no evidence of the presence of spores of *Endothia parasitica* in the air. In the afternoon of August 27, however, two thunder storms occurred, in consequence of which the regular routine was departed from. Tables II and III show the outcome of the tests of this date. After the first storm two sets of plates were exposed in the course of an hour and a half. Two out of the second set yielded colonies of *E. parasitica*. Since the ascospore-trap tests (Tables X and XVII) did not give evidences of expulsion occurring when the first five plates were exposed, negative results were to be expected in those plates, and it is not surprising that only two out of the second set of seven plates yielded colonies of *E. parasitica* when the 19 ascospore traps examined for this particular period showed evidence of expulsion from only one perithecium. The meagerness of these results is partially accounted for by the small amount of rain, rapid drying, and the fact that the perithecia had hardly been wet a sufficient length of time.

TABLE III.—*Record of exposure plates made on August 27, 1913, at West Chester, Pa.*

BEFORE RAIN.

| Plate No. | Time. | Length of exposure. | Station No. | Wind. Direction. | Wind. Miles per hour. | Number of bacteria and yeasts. | Total number of fungi. | Number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| | | *Minutes.* | | | | | | |
| 4359 | 8.35 a. m..... | 4 | 1 | SW. | 1.6 | 2 | 7 | 0 |
| 4360 | 9.09 a. m..... | 5 | 3 | SW. | 1.6 | 1 | 8 | 0 |
| 4361 | 9.32 a. m..... | 5 | 1 | SW. | 1.6 | 1 | 6 | 0 |
| 4362 | 10.03 a. m.... | 5 | 13 | SW. | 1.6 | 0 | 6 | 0 |
| 4363 | 10.29 a. m.... | 6 | 14 | SW. | 1.6 | 1 | 7 | 0 |
| 4364 | 10.57 a. m.... | 5 | 3 | W. | 1.6 | 3 | 9 | 0 |
| 4365 | 11.34 a. m.... | 5½ | 1 | W. | 1.7 | 2 | 19 | 0 |
| 4366 | 11.57 a. m.... | 5 | 1 | W. | 1.7 | 0 | 15 | 0 |
| 4367 | 1.16 p. m..... | 5 | 1 | W. | 1.7 | 0 | 22 | 0 |
| 4368 | 1.39 p. m..... | 5 | 13 | W. | 1.7 | 0 | 33 | 0 |
| 4369 | 2.09 p. m..... | 5½ | 13 | W. | 1.7 | 1 | 13 | 0 |
| 4370 | 2.45 p. m..... | 5 | 3 | W. | 1.7 | 1 | 7 | 0 |
| RAIN NO. 1 (0.12 INCH, 3.15 TO 3.28 P. M.) | | | | | | | | |
| 4371 | 3.48 p. m..... | 7 | 3 | W. | 2.4 | 1 | 12 | 0 |
| 4372 | 3.50 p. m..... | 6½ | 15 | W. | 2.4 | 0 | 10 | 0 |
| 4373 | 3.49 p. m..... | 6⅝ | 13 | W. | 2.4 | 0 | 17 | 0 |
| 4374 | 3.48 p. m..... | 6¾ | 1 | W. | 2.4 | 0 | 32 | 0 |
| 4375 | 3.51 p. m..... | 7 | 8 | W. | 2.4 | 0 | 9 | 0 |
| 4376 | 4.10 p. m..... | 9⅓ | 6 | W. | Trace. | 0 | 7 | 1 |
| 4377 | 4.12 p. m..... | 8⅔ | 11 | W. | Trace. | 0 | 8 | 0 |
| 4378 | 4.14 p. m..... | 8⅕ | 3 | W. | Trace. | 2 | 15 | 0 |
| 4379 | 4.15 p. m..... | 8 | 16 | W. | Trace. | 0 | 11 | 0 |
| 4380 | 4.17 p. m..... | 5⅝ | 15 | W. | Trace. | 1 | 9 | 0 |
| 4381 | 4.40 p. m..... | 4 | 3 | W. | Trace. | 0 | 4 | 0 |
| 4382 | 5.12 p. m..... | 6 | 3 | W. | Trace. | 1 | 13 | 3 |

TABLE III.—*Record of exposure plates made on August 27, 1913, at West Chester, Pa.*—Continued

RAIN NO. 2 (0.055 INCH, 5.35 TO 5.50 P. M.)

| Plate No. | Time. | Length of exposure. | Station No. | Wind. Direction. | Wind. Miles per hour. | Number of bacteria and yeasts. | Total number of fungi. | Number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| | | *Minutes.* | | | | | | |
| 4383 | 6.08 p. m. . . . . | 6½ | 3 | NW. | 1.2 | 0 | 30 | 16 |
| 4384 | 6.10 p. m. . . . . | 6 | 1 | NW. | 1.2 | 0 | 25 | 21 |
| 4385 | 6.12 p. m. . . . . | 8 | 16 | NW. | 1.2 | 2 | 70 | 33 |
| 4386 | 6.23 p. m. . . . . | 5 | 6 | N. | .7 | 0 | 25 | 20 |

The second shower on August 27 took place late in the afternoon, and though the precipitation was light, the cumulative effect of this rain upon that of the preceding one caused abundant expulsion of ascospores. The four plates exposed within about half an hour after this shower yielded colonies of *E. parasitica* in such numbers as to prove beyond doubt that the ascospores were at that time very prevalent in the air. The ascospore-trap tests for this period (Tables X and XVII) showed that, although out of the 14 examined only 1 bore any evidence of spore expulsion during the first 15 minutes after the cessation of the rain, 12 out of 14 showed expulsion of ascospores during the time in which the plate exposures were made. The sun had gone down, and the weather conditions following this storm were not conducive to the rapid drying of the bark. The results of this date were the first evidence secured which indicated beyond doubt that ascospores of *Endothia parasitica* are disseminated by wind under natural conditions.

During the dry, hot weather of August 28 evidently no spores of *Endothia parasitica* were present in the air, nor were any detected on August 29. On this date the humidity was high and cloudiness prevailed, accompanied by traces of rain insufficient to cause ascospore expulsion. As there was a rainfall of 1.10 inches in the evening of August 29, several plates were exposed in rapid succession the next morning, but no spores were caught. This failure is attributed to the fact that once more too long a time had passed since the rain ceased, and spore expulsion, though probably abundant in the night, had no doubt ceased long before the first exposures were made. Throughout the following week there was no rain, and no ascopore expulsion occurred at any time.

For the night previous to September 8 a rainfall of 0.37 inch was recorded, the time of cessation being prior to 1.30 a. m. The ascopore traps gave evidence of plentiful spore expulsion. Between 6.27 and 8 a. m. eight exposures were made before the sun had dried the vegetation and while the bark was still wet in places. Three of these plates yielded colonies of *Endothia parasitica*, and as two of them were exposed at stations more or less in the open, it would seem that ascospores were at that time prevalent in the air to some extent. The third plate and also five others exposed at later intervals during the day each yielded one colony of *E. parasitica*.

Of the plates exposed on September 9, a dry, hot day, two in the morning also yielded one colony each of *Endothia parasitica*. During the dry weather of September 10 and 11 negative results were obtained.

Cloudiness prevailed on September 12, with traces of rain insufficient to cause spore expulsion. In the night less than one-tenth of an inch of rain fell, and subsequent examination of the ascospore traps showed that very light ascospore expulsion had occurred. Five plates were exposed before 8 o'clock the next morning, but the bark was dry at the time and no spores were obtained in any of the plates exposed that day. Three days of clear, hot weather followed, and no spores were caught. Of the plates exposed on September 17, two yielded one colony each of the fungus.

In the evening of September 17 a series of rains began, occurring usually in the night. Our most important positive results were obtained from tests made following these rains. Tables II and IV give the results of the plates exposed on September 18. Although the rain ceased before 4 a. m., a heavy fog prevailed in the early morning, there was only a trace of wind, and it was more or less cloudy all day. Because of these conditions the bark of the trees was slow in drying, and examination of the ascospore traps (Tables XI and XVII) showed that abundant spore expulsion had occurred in the night and was still in progress while the first four exposure plates were made. A few of the traps gave evidences of the continuation of expulsion during the time in which the next 11 plates were exposed. Six of these yielded colonies of *Endothia parasitica* in varying numbers, and two exposed much later in the day also showed one colony each.

TABLE IV.—*Record of exposure plates made on September 18, 1913, at West Chester, Pa.*[a]

| Plate No. | Time. | Length of exposure. | Station No. | Wind. | | Number of bacteria and yeasts. | Total number of fungi. | Number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| | | | | Direction. | Miles per hour. | | | |
| | | *Minutes.* | | | | | | |
| 4787 | 5.51 a. m. | 9¾ | 3 | NW. | Trace. | 4 | 20 | 11 |
| 4788 | 5.54 a. m. | 10 | 15 | NW. | Trace. | 6 | 58 | 16 |
| 4789 | 6.00 a. m. | 10 | 6 | NW. | Trace. | 6 | 39 | 9 |
| 4790 | 6.19 a. m. | 13 | 1 | NW. | Trace. | (b) | 59 | 4 |
| 4791 | 6.35 a. m. | 11 | 11 | NW. | Trace. | (b) | 67 | 5 |
| 4792 | 6.48 a. m. | 11 | 3 | NW. | Trace. | 1 | 16 | 2 |
| 4793 | 6.54 a. m. | 12 | 4 | NW. | Trace. | (b) | 32 | 2 |
| 4794 | 7.02 a. m. | 10½ | 16 | NW. | Trace. | (b) | 23 | 0 |
| 4795 | 7.25 a. m. | 11 | 3 | NW. | Trace. | (b) | 30 | 2 |
| 4796 | 7.34 a. m. | 11 | 13 | NW. | 0.4 | 3 | 8 | 1 |
| 4797 | 7.58 a. m. | 10¼ | 15 | W. | .7 | 8 | 11 | 1 |
| 4798 | 8.04 a. m. | 10¾ | 9 | W. | .7 | 0 | 2 | 0 |
| 4799 | 8.29 a. m. | 10 | 17 | W. | .7 | 0 | 1 | 0 |
| 5000 | 9.20 a. m. | 12¼ | 1 | W. | 1.1 | 0 | 8 | 0 |
| 5001 | 9.49 a. m. | 11 | 3 | W. | 1.8 | 0 | 25 | 0 |
| 5002 | 10.21 a. m. | 10¼ | 6 | W. | 2.8 | 5 | 10 | 0 |
| 5003 | 10.54 a. m. | 12 | 15 | W. | 2.8 | 1 | 19 | 0 |
| 5004 | 11.25 a. m. | 11 | 33 | W. | 3.7 | 0 | 12 | 0 |
| 5005 | 11.51 a. m. | 11½ | 3 | W. | 3.7 | 3 | 13 | 1 |
| 5006 | 1.14 p. m. | 13½ | 26 | W. | 2.2 | 1 | 17 | 0 |
| 5007 | 1.44 p. m. | 10¼ | 21 | W. | 2.8 | 0 | 7 | 0 |
| 5008 | 2.15 p. m. | 10½ | 22 | W. | 1.8 | 1 | 10 | 0 |
| 5009 | 2.46 p. m. | 15 | 6 | W. | 1.2 | 1 | 12 | 1 |
| 5010 | 3.19 p. m. | 11 | 33 | W. | 1.2 | 0 | 12 | 0 |
| 5011 | 3.45 p. m. | 11 | 21 | W. | .9 | 1 | 16 | 0 |
| 5012 | 4.16 p. m. | 13½ | 25 | W. | .9 | 0 | 1 | 0 |
| 5013 | 4.44 p. m. | 11½ | 23 | W. | .2 | 4 | 21 | 0 |
| 5014 | 5.23 p. m. | 10¾ | 1 | W. | .1 | 0 | 3 | 0 |

[a] Rainfall, night previous, 0.26 inch. Time of cessation, prior to 4 a. m. [b] Numerous.

In the night of September 18 a rain of 0.68 inch was recorded, the time of cessation being the next morning before 3.45. Fog again prevailed all day September 19 and a noticeable spray fell until 5.38 a. m. and began again after 2.41 p. m. Ascospore-trap tests (Tables XII and XVII) showed that in 10 out of the 17 traps examined there was spore expulsion after 7.35 a. m. Of the eight plates exposed prior to this time but two yielded colonies of this Endothia. The first three plates were exposed in an open field at considerable distances from the trees (fig. 2, stations 41, 42, and 43) in the same direction toward which the wind was blowing, but no spores of *E. parasitica* were caught. These negative results may be accounted for by the action of the falling mist. Later in the day four exposures at various intervals yielded one colony each of the chestnut-blight fungus.

TABLE V.—*Record of exposure plates made on September 20, 1913, at West Chester, Pa.*[a]

| Plate No. | Time. | Length of exposure. | Station No. | Wind. | | Number of bacteria and yeasts. | Total number of fungi. | Number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| | | | | Direction. | Miles per hour. | | | |
| | | *Minutes.* | | | | | | |
| 5041 | 5.50 a. m. | 16 | 44 | NE. | 2.6 | 0 | 12 | 10 |
| 5042 | 5.55 a. m. | 13 | 45 | NE. | 2.6 | 0 | 9 | 7 |
| 5043 | 5.56 a. m. | 14 | 46 | NE. | 2.6 | 0 | 10 | 4 |
| 5044 | 6.03 a. m. | 12¾ | 51 | NE. | 2.6 | 0 | 23 | 22 |
| 5045 | 6.14 a. m. | 12¼ | 47 | NE. | 2.6 | 0 | 14 | 11 |
| 5046 | 6.18 a. m. | 15 | 48 | NE. | 2.6 | 0 | 9 | 7 |
| 5047 | 6.20 a. m. | 15 | 49 | NE. | 2.6 | 0 | 11 | 10 |
| 5048 | 6.40 a. m. | 13½ | 11 | ENE. | 2.6 | 0 | 6 | 6 |
| 5049 | 7.07 a. m. | 11½ | 1 | ENE. | 2.6 | 0 | 9 | 7 |
| 5050 | 7.32 a. m. | 13 | 9 | ENE. | 2.6 | 0 | 6 | 3 |
| 5051 | 7.40 a. m. | 16¾ | 50 | ENE. | 2.6 | 0 | 6 | 1 |
| 5052 | 8.24 a. m. | 10 | 6 | ENE. | 2.5 | 0 | 1 | 1 |
| 5053 | 9.09 a. m. | 13½ | 8 | ENE. | 2.5 | 0 | 1 | 1 |
| 5054 | 9.37 a. m. | 14½ | 27 | ENE. | 3.0 | 5 | 11 | 0 |
| 5055 | 10.01 a. m. | 14¾ | 6 | ENE. | 2.4 | 0 | 2 | 0 |
| 5056 | 10.32 a. m. | 9¼ | 10 | E. | 2.4 | 0 | 3 | 0 |
| 5057 | 11.09 a. m. | 10 | 29 | E. | 2.6 | 0 | 12 | 0 |
| 5058 | 11.44 a. m. | 11¼ | 26 | E. | 2.6 | 0 | 6 | 0 |
| 5059 | 12.08 p. m. | 10 | 10 | E. | 2.7 | 0 | 2 | 0 |
| 5060 | 1.05 p. m. | 14½ | 30 | E. | 2.7 | 0 | 2 | 0 |
| 5061 | 1.35 p. m. | 13½ | 11 | E. | 2.5 | 0 | 1 | 0 |
| 5062 | 2.08 p. m. | 14¾ | 10 | E. | 2.5 | 0 | 19 | 0 |
| 5063 | 2.36 p. m. | 15½ | 8 | E. | 2.7 | 2 | 4 | 0 |
| 5064 | 3.09 p. m. | 13¼ | 9 | E. | 2.7 | 0 | 1 | 0 |
| 5065 | 3.42 p. m. | 14 | 29 | E. | 2.7 | 0 | 2 | 0 |
| 5066 | 4.06 p. m. | 18 | 23 | E. | 2.3 | 0 | 4 | 0 |
| 5067 | 4.41 p. m. | 12¼ | 12 | ESE. | 2.7 | 0 | 4 | 0 |
| 5068 | 5.25 p. m. | 12 | 12 | SE. | 2.7 | 3 | 4 | 0 |

[a] Rainfall, night previous, 0.09 inch. Time of cessation, 3.25 to 3.55 a. m.

While there was but 0.09 inch of rain in the night of September 19, very important results were obtained on September 20. Tables II and V give the results secured. Fog prevailed during the entire day, and the bark on the trees dried very slowly. Examination of the ascospore traps to determine the duration of spore expulsion (Tables XIII and XVII) showed that in 8 out of 19 the perithecia were active after 9.18 a. m.

All of the 13 plates exposed previous to this time yielded colonies of *Endothia parasitica*. Eight of these exposures were made in the open field at varying distances south and west of the plot of diseased trees (fig. 2), the wind being from the northeast. The distance relations brought out by these tests are discussed later. Although five ascospore traps showed evidences of the occurrence of spore expulsion after 10.07 a. m., no colonies of this fungus appeared in any of the plates exposed after 9.23 a. m. This indicates that spores were evidently not sufficiently numerous in the air after that time to be detected by the exposure-plate method.

The results obtained on September 21, as shown in Tables II and VI, bring out again the direct relation of rain to wind dissemination. Two plates exposed during a 16-minute interval between showers in the early morning yielded colonies of *Endothia parasitica* in such numbers as to prove without doubt that ascospores were very prevalent in the air at that time. After the second rain, ending at 8.20 a. m., only the five plates exposed within an hour after its cessation yielded colonies of *E. parasitica*, even though 14 out of the 21 ascospore traps examined showed that considerable spore expulsion had taken place after 10 a. m. (Table XI). However, a south wind of increasing velocity prevailed, and at 9.21 the sun appeared, causing a marked rise in temperature, so that the bark dried very rapidly after that time. Furthermore, the higher wind may also have dispersed and scattered the fewer spores expelled thereafter to such an extent that none happened to fall into the exposed plates.

TABLE VI.—*Record of exposure plates made on September 21, 1913, at West Chester, Pa.*

RAIN NO. 1 (ABOUT 0.35 INCH, CEASED 6.24 A. M.)

| Plate No. | Time. | Length of exposure. | Station No. | Wind. Direction. | Wind. Miles per hour. | Number of bacteria and yeasts. | Total number of fungi. | Number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| | | *Minutes.* | | | | | | |
| 5069 | 6.23 a. m. | $14\frac{1}{4}$ | 12 | SSE. | 2.6 | ([a]) | 30 | 12 |
| 5070 | 6.30 a. m. | $10\frac{1}{2}$ | 13 | SSE. | 2.6 | ([a]) | 50 | 20 |

RAIN NO. 2 (ABOUT 0.08 INCH, 6.40 TO 8.20 A. M.)

| Plate No. | Time. | Length of exposure. | Station No. | Wind. Direction. | Wind. Miles per hour. | Number of bacteria and yeasts. | Total number of fungi. | Number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| 5071 | 8.35 a. m. | 20 | 13 | SSE. | 2.6 | ([b]) | 70 | 62 |
| 5072 | 8.37 a. m. | 19 | 12 | SSE. | 2.6 | 2 | 28 | 24 |
| 5073 | 9.04 a. m. | $13\frac{1}{2}$ | 13 | SSE. | 3.1 | 1 | 21 | 19 |
| 5074 | 9.16 a. m. | $15\frac{1}{4}$ | 19 | S. | 3.1 | 1 | 5 | 1 |
| 5075 | 9.21 a. m. | 12 | 11 | S. | 3.1 | 0 | 9 | 2 |
| 5076 | 9.40 a. m. | 15 | 13 | S. | 3.1 | 3 | 37 | 0 |
| 5077 | 9.43 a. m. | $13\frac{1}{2}$ | 37 | S. | 3.1 | 1 | 18 | 0 |
| 5078 | 10.25 a. m. | 15 | 13 | SSW. | 5.8 | 3 | 30 | 0 |
| 5079 | 10.28 a. m. | 16 | 19 | SSW. | 5.8 | 12 | 80 | 0 |
| 5080 | 10.46 a. m. | 10 | 37 | SSW. | 5.8 | 0 | 21 | 0 |
| 5081 | 11.00 a. m. | 12 | 14 | SSW. | 5.8 | 6 | 44 | 0 |
| 5082 | 11.07 a. m. | $13\frac{1}{2}$ | 37 | SSW. | 5.8 | 1 | 28 | 0 |
| 5083 | 11.48 a. m. | $16\frac{1}{2}$ | 12 | SSW. | 5.8 | 5 | 98 | 0 |
| 5084 | 1.19 p. m. | $15\frac{1}{2}$ | 13 | S. | 5.6 | 0 | 26 | 0 |

[a] Numerous. [b] Few.

TABLE VI.—*Record of exposure plates made on September 21, 1913, at West Chester, Pa.*—Continued

RAIN NO. 3 (ABOUT 0.03 INCH, 1.43 TO 2.11 P. M.)

| Plate No. | Time. | Length of exposure. | Station No. | Wind. Direction. | Wind. Miles per hour. | Number of bacteria and yeasts. | Total number of fungi. | Number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| | | *Minutes.* | | | | | | |
| 5085 | 2.20 p. m..... | 3½ | 13 | S. | 6.2 | 8 | 160 | 0 |

RAIN NO. 4 (ABOUT 0.04 INCH, 2.20 TO 2.50 P. M.)

| Plate No. | Time. | Length of exposure. | Station No. | Wind. Direction. | Wind. Miles per hour. | Number of bacteria and yeasts. | Total number of fungi. | Number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| 5086 | 2.53 p. m..... | 15¾ | 13 | S. | 6.2 | 0 | 144 | 0 |
| 5087 | 3.10 p. m..... | 14½ | 12 | S. | 6.2 | 0 | 35 | 20 |

RAIN NO. 5 (ABOUT 0.03 INCH, 3.26 TO 4.07 P. M.)

| Plate No. | Time. | Length of exposure. | Station No. | Wind. Direction. | Wind. Miles per hour. | Number of bacteria and yeasts. | Total number of fungi. | Number of colonies of Endothia parasitica. |
|---|---|---|---|---|---|---|---|---|
| 5086A | 4.08 p. m..... | 19¾ | 13 | S. | 8.0 | 10 | 58 | 0 |
| 5087A | 4.17 p. m..... | 14 | 37 | S. | 8.0 | 3 | 50 | 0 |
| 5088 | 4.35 p. m..... | 10 | 11 | S. | 8.0 | 4 | 45 | 0 |
| 5089 | 4.55 p. m..... | 15 | 12 | S. | 8.0 | 6 | 42 | 0 |
| 5090 | 5.16 p. m..... | 17½ | 11 | S. | 8.0 | 3 | 38 | 0 |
| 5091 | 5.33 p. m..... | 11 | 10 | S. | 8.0 | 5 | 20 | 0 |

In the afternoon of the same day three light showers occurred, and one plate exposed in the interval after the second of these caught 20 ascospores. After the first of these showers the exposure was cut too short by the recurrence of rain to give a reliable test. It will be seen that none of the six plates exposed during the 1 hour and 36 minutes after the last shower yielded colonies of *Endothia parasitica*, despite the fact that 6 out of 11 ascospore traps examined (Tables XIV and XVII) gave evidence that expulsion had occurred during that period. In explanation it may be stated that the wind had attained a higher velocity at this time and was blowing quite briskly in the open. It is readily conceivable that with such a wind the spores as they were expelled might have been transported with such speed and their numbers dissipated so rapidly that none chanced to fall on the rather small area represented by the exposure plates.

A rather heavy rainfall was recorded on the night of September 21, but it ceased before 12.45 a. m. Examination of the ascospore traps showed that there was abundant spore expulsion during the night, and 5 out of 21 traps gave evidences of the occurrence of expulsion after 7.30 a. m., on September 22 (Tables XV and XVII). Of the 13 plates exposed between 5.56 a. m. and 7.35 a. m. but 2 yielded positive results (Table II). No spores were caught in any of the plates exposed thereafter, even though two ascospore traps bore evidences of the occurrence of light expulsion after 11.24 a. m. The meager results obtained on this date are no doubt due to the long period of time intervening since the cessation of rain the night before.

Clear, hot weather prevailed during September 22 and 23, and no spores were caught.

The relation of the time elapsed since the cessation of rain to the prevalence of ascospores in the air among diseased trees is shown in Table VII.

TABLE VII.—*Relation of the time elapsed since the cessation of rain to the number of spores falling on an area of 1 square foot per minute in 1913 at West Chester, Pa.*

PLATES EXPOSED ON SEPTEMBER 18, 1913

| No. of plate. | Time elapsed since cessation of rain. | Number of colonies of Endothia parasitica. | Number of spores of Endothia parasitica falling on an area of 1 square foot per minute. |
|---|---|---|---|
| | *H. m.* | | |
| 4787 | 1 51 | 11 | 15.74 |
| 4788 | 1 54 | 16 | 22.32 |
| 4789 | 2 .. | 9 | 12.55 |
| 4790 | 2 19 | 4 | 4.29 |
| 4791 | 2 35 | 5 | 6.34 |
| 4792 | 2 48 | 2 | 2.53 |
| 4793 | 2 54 | 2 | 2.32 |
| 4795 | 3 25 | 2 | 2.53 |
| 4796 | 3 34 | 1 | 1.27 |
| 4797 | 3 58 | 1 | 1.36 |
| 4798 | 4 4 | 0 | 0 |
| 4799 | 4 29 | 0 | 0 |
| PLATES EXPOSED ON SEPTEMBER 21, 1913 | | | |
| 5071 | 15 | 62 | 43.24 |
| 5072 | 17 | 24 | 17.62 |
| 5073 | 44 | 19 | 19.64 |
| 5074 | 56 | 1 | 0.91 |
| 5075 | 1 1 | 2 | 2.32 |
| 5076 | 1 20 | 0 | 0 |
| 5077 | 1 23 | 0 | 0 |
| 5078 | 2 5 | 0 | 0 |

An examination of these tables shows that on September 18 the spore content of the air decreased more or less gradually during the third and fourth hours after the rain, while on September 21 the spore content decreased very abruptly and no spores were obtained after the first hour following the cessation of the rain. The duration and the abundance of the ascospore expulsion on these dates (Table XI) are seen to have differed likewise, and a comparison of the weather conditions gives the probable explanation, since it was calm and foggy on the 18th and hot and sunny with a brisk wind just following the rain of the 21st. Conditions following the rain on the 18th were such as to prevent rapid drying of the bark, so that spore expulsion continued during a much longer time than on the 21st, when the bark dried rapidly. Furthermore, the brisk wind of September 21 would tend to disperse the spores very rapidly, whereas the comparative calm of September 18 would be favorable to a more prolonged prevalence in the air near their source. In this regard

it should also be noted that on September 20 (Table II), when foggy weather followed the rain, spores were prevalent in the air during at least five hours after the rain had ceased.

A glance at the figures representing the number of spores falling each minute on a surface equal to 1 square foot shows that during periods of one to four or more hours after a rain—in other words, during such time as expulsion continues—healthy trees among diseased ones would be subject to infection, since some of the ascospores would find lodgment upon exposed parts of trunks and branches.

The results obtained in the early morning of September 20 by making exposures in an open field at varying distances from the principal source of spores (figs. 2 and 3) are presented in Table VIII.

TABLE VIII.—*Relation of distance from source of spores to number of spores falling on an area of 1 square foot per minute in 1913 at West Chester, Pa.*[a]

| Plate No. | Time. | Distance from source of spores. | Number of spores falling on an area of 1 square foot per minute. |
|---|---|---|---|
| | | *Feet.* | |
| 5044 | 6.03 a. m. | 27 | 24.07 |
| 5045 | 6.14 a. m. | 85 | 12.52 |
| 5046 | 6.18 a. m. | 180 | 6.51 |
| 5042 | 5.55 a. m. | 266 | 7.51 |
| 5047 | 6.20 a. m. | 409 | 9.30 |
| 5041 | 5.50 a. m. | 414 | 8.71 |

[a] Plates exposed on Sept. 20, 1913.

These exposures, all made within about half an hour and in the same general direction from the plot of diseased trees—i. e., the direction toward which the wind was blowing—show that in a general way the number of spores falling upon equal surfaces in equal intervals of time decreases as the distance from the source of spores is increased. The fact alone that in an open field at rather long distances from diseased trees ascospores were prevalent in the air to such an extent that every minute from 6 to 24 spores were settling upon a surface equal to 1 square foot (Pl. LXIII, figs. 1 and 2) indicates that at such a time many opportunities would be offered for exposed parts of undiseased trees at considerable distances from diseased ones to become infected by wind-borne ascospores.

Furthermore, these results show that the maximum distance over which ascospores might be transported by the wind was by no means obtained, and the large numbers found at the longest distances in this experiment, given in Table VIII, when a light wind prevailed, indicate that even with a relatively light wind ascospores are probably conveyed distances far greater than these.

In a consideration of the exposure plates yielding the high numbers of colonies of *Endothia parasitica* it is interesting to note the relatively large proportion of the spore content of the air formed by ascospores of this fungus at certain times (Table IX).

TABLE IX.—*Percentage of the number of spores of Endothia parasitica to the total spore content of the air, as shown by exposure-plate tests on chestnut-bark agar in 1913 at West Chester, Pa.*

| Plate No. | Date. | Total number of fungous colonies. | Number of colonies of Endothia parasitica. | Percentage of colonies of Endothia parasitica to total spore content of air. | Plate No. | Date. | Total number of fungous colonies. | Number of colonies of Endothia parasitica. | Percentage of colonies of Endothia parasitica to total spore content of air. |
|---|---|---|---|---|---|---|---|---|---|
| 4383 | Aug. 27 | 30 | 16 | 53 | 5046 | Sept. 20 | 9 | 7 | 77 |
| 4384 | ...do | 25 | 21 | 84 | 5047 | ...do | 11 | 10 | 91 |
| 4385 | ...do | 70 | 33 | 47 | 5048 | ...do | 6 | 6 | 100 |
| 4386 | ...do | 25 | 20 | 80 | 5049 | ...do | 9 | 7 | 77 |
| 4787 | Sept. 18 | 20 | 11 | 55 | 5050 | ...do | 6 | 3 | 50 |
| 5041 | Sept. 20 | 12 | 10 | 83 | 5069 | Sept. 21 | 30 | 12 | 40 |
| 5042 | ...do | 9 | 7 | 77 | 5070 | ...do | 50 | 20 | 40 |
| 5043 | ...do | 10 | 4 | 40 | 5071 | ...do | 70 | 62 | 88 |
| 5044 | ...do | 23 | 22 | 95 | 5072 | ...do | 28 | 24 | 85 |
| 5045 | ...do | 14 | 11 | 78 | 5073 | ...do | 21 | 19 | 90 |

In connection with these figures it should be borne in mind that the fungi represented are such as will grow only on chestnut-bark agar. Taking into consideration, however, the relatively large numbers of other fungi ordinarily developing in the exposure plates (Table I), it is a noteworthy fact that at certain periods when ascospore expulsion was in progress the spores of this one species should constitute from 40 to 100 per cent of the total spore content of the air.

Since these plates were exposed not long after a rain, a possible explanation suggested is that spores of other fungi were washed from the air by the rain and the supply had not yet been replenished, whereas conditions were very favorable to the abundant expulsion of ascospores of *Endothia parasitica*. It has also been suspected that certain types other than this fungus which were often found in plates exposed at such times represented other ascomycetous fungi the spores of which had just been expelled.

## SUMMARY OF EXPOSURE-PLATE TESTS

In all of the exposure plates yielding colonies of *Endothia parasitica* it was determined from the time of appearance of these colonies that all originated from ascospores. Therefore we may safely state at the outset that under the conditions of the tests little or no wind dissemination of pycnospores occurred.

By comparison with ascospore-trap tests it is evident that ascospores of *Endothia parasitica* were caught in the exposure plates in numbers and at some distances from trees only during certain periods following rains when ascospore expulsion was in progress. The possible exception occurred on the morning of September 8, when no series of observations was made on the ascopore traps.

As the occurrence of ascospores in the air in considerable numbers is the prime requisite for wind dissemination and as ascospore expulsion

occurs only when the perithecia-bearing bark has been wet by rains, the following facts are presented to show that wind dissemination is directly dependent upon weather conditions causing spore expulsion.

Of the total number of 756 plates exposed during these tests 95 were exposed while ascospore expulsion was known to have been in progress, and of these, 41 yielded colonies of *Endothia parasitica*. Of the remaining 661 plates exposed at other times than those noted above, but 23 yielded colonies of *E. parasitica*, and 14 of these were exposed within 12 hours after expulsion was known to have occurred.

To bring out in a more striking manner the relation of rain to wind dissemination, it is worthy of note that out of a total of 427 ascospores of *Endothia parasitica* caught in the exposed plates 402, or 94 per cent, were caught in plates exposed while spore expulsion was known to have been in progress, and of the remaining 25 spores 3 were caught within 5 hours after the cessation of a rain (Sept. 8) and 12 more were caught within 12 hours after ascospore expulsion was known to have occurred. This leaves but 10 out of 427 spores, or 2.3 per cent, seeming to be stray ascospores bearing no relation to a rain.

As to the origin of the 22 colonies of *Endothia parasitica* appearing in plates exposed when spore expulsion was known not to be in progress (see Table II), the following points are cited to prove that they originated from stray ascospores which, after expulsion, lodged on near-by or, perhaps, distant trunks, limbs, or leaves and were subsequently loosened by the mechanical action of some agency.

1. All but one of the 21 plates containing these colonies yielded only a single colony of *Endothia parasitica* each.

2. In one colony a fragment of bark was visible at its center.

3. All except one of these spores were caught at stations more or less overhung by branches of diseased trees, and all except three were caught on stumps surrounded by sprouts.

4. Only 1 out of 192 plates exposed at unsheltered stations when expulsion was not in progress yielded a colony of *Endothia parasitica*.

If these had been stray spores that were still floating in the air since expulsion, they would have fallen just as frequently into plates exposed out in the open at unsheltered stations. During a period of ascospore expulsion following a rain it seems probable that the spores would not all be swept away by air currents but that some few would find lodgment upon near-by leaves and branches. Such lodgment is especially likely to take place if there is no noticeable wind when expulsion is in progress. Thus, it seems quite probable that the colonies obtained when perithecia were not active originated from spores dislodged from either healthy or diseased parts of trees more or less overhanging the plates.

Unless attached to a bark fragment, the path of these spores in falling would not necessarily approach the vertical, and such spores might be transported by the wind just as readily as though they were freshly expelled. This explains, perhaps, why one spore was caught in plate No. 5037, exposed 27 feet from the nearest chestnut tree. The probable reason, then, why, with this exception, such stray spores were caught only under trees is that the rareness of their occurrence in the air prevented their detection elsewhere than in very close proximity to their place of temporary lodgment, since with the exposure-plate method the chance of detecting these spores decreases very rapidly as the distance from their source is increased.

Obviously no exposures could be made during a rain, but ascospore-trap examinations have shown that abundant spore expulsion may occur during the actual fall of the rain. It is evident, however, that at such a time wind dissemination would be reduced to a minimum, because the spores upon expulsion would soon be washed to the ground or to near-by bark or foliage.

Therefore, under the conditions of our tests it can be said that, with the exception of the few stray ascospores loosened from temporary lodgment, wind dissemination of *Endothia parasitica* occurs only during certain periods after rains, when ascospore expulsion is in progress.

## ASCOSPORE-TRAP TESTS

In order to detect ascospore expulsion whenever it occurred, use was made of what we have termed "ascospore traps." An ascospore trap consisted of a glass object slide held in place over perithecial pustules on the bark of a diseased tree by means of a wooden bracket either above or below the slide (Pl. LXIV, figs. 1 and 2). The slide was wedged firmly into a slot in the bracket so as to be suspended about one-eighth of an inch or less from the papillæ underneath. These traps were placed on lesions of various ages on trees more or less scattered throughout the experimental plot (fig. 1).

As the ascospores of *Endothia parasitica* are expelled they adhere to the glass, and the spores expelled from each ostiole usually form a definite "spot," so that the number of spots on the slide represents the number of perithecia in the area underneath which have expelled spores.

During the progress of the work on wind dissemination, it was found possible by means of these traps not only to detect the occurrence of ascospore expulsion but to determine even with some degree of accuracy the exact duration of perithecial activity.

As has been brought out in the discussion of the exposure-plate tests, the occurrence of ascospores in the air in numbers is directly dependent upon the continuation of their expulsion after a rain has ceased. The duration of expulsion becomes, therefore, an essential factor in determining the period during which wind dissemination may occur.

In making this determination the method of procedure was as follows: Out of the total number of 69 ascospore traps usually about 20 were selected, representing areas of vigorous perithecia where previous experience indicated that abundant expulsion was most likely to occur. The slides from these traps were collected as soon as possible after the rain and were replaced with clean slides. Then, after a convenient interval, this second set of slides was collected and replaced with clean ones. This operation was repeated at intervals of several minutes to several hours until none of the slides bore spots of expelled ascospores.

Several series of trap collections were usually made after each rain, and a subsequent examination of each slide revealed whether or not any expulsion had occurred under that trap in the period during which that particular slide had been in place on the tree. Although usually visible to the unaided eye, an examination with a hand lens was often necessary to detect very faint or very diffuse spots of ascospores on the slides.

The detailed results for September 20 are given to show the behavior of individual traps (Table X). The results given in the summary for the other dates were obtained in a similar manner and the individual records will therefore be omitted.

TABLE X.—*Record of ascospore-trap collections on September 20, 1913, at West Chester, Pa.*[a]

| Time from cessation of rain to— | | Number of perithecia expelling ascospores between times stated. | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replacing of slides. | Collection of slides. | Trap No. 59. | Trap No. 58. | Trap No. 60. | Trap No. 62. | Trap No. 63. | Trap No. 64. | Trap No. 65. | Trap No. 68. | Trap No. 69. | Trap No. 74. | Trap No. 75. | Trap No. 72. | Trap No. 14. | Trap No. 15. | Trap No. 30. | Trap No. 40. | Trap No. 45. | Trap No. 4. | Trap No. 50. |
| .......... | $2^h48^m$ to $3^h10^m$ | 44 | 22 | 118 | 73 | 79 | 53 | 40+ | 14 | 238+ | 82 | 77 | 2 | 7 | 13 | 8 | 5 | 0 | 0 | 0 |
| $2^h48^m$ to $3^h10^m$ | $4^h5^m$ to $4^h27^m$ | 23 | 7 | 67 | 66 | 43 | 5 | 23 | 0 | 101 | 53 | 34 | 1 | 3 | 2 | 1 | 4 | 0 | 0 | 0 |
| $4^h5^m$ to $4^h27^m$ | $5^h23^m$ to $5^h41^m$ | 6 | 4 | 19 | 17 | 15 | 1 | 9 | 0 | 22 | 30 | 4 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 |
| $5^h23^m$ to $5^h41^m$ | $6^h12^m$ to $6^h33^m$ | 0 | 1 | 0 | 8 | 1 | 1 | 1 | 0 | 11 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| $6^h12^m$ to $6^h33^m$ | $6^h53^m$ to $7^h11^m$ | 0 | 0 | 0 | 5 | 0 | 0 | 0 | .. | 4 | 0 | .. | .. | 1 | 0 | .. | .. | 0 | 0 | 0 |
| $6^h53^m$ to $7^h11^m$ | $7^h35^m$ to $7^h47^m$ | .. | .. | .. | 3 | 0 | .. | .. | .. | 2 | .. | .. | .. | 0 | 0 | .. | .. | 0 | 0 | .. |
| $7^h35^m$ to $7^h47^m$ | $10^h34^m$ to $11^h31^m$ | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | .. | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| $10^h34^m$ to $11^h31^m$ | $13^h14^m$ to $13^h38^m$ | .. | 9 | .. | .. | .. | .. | .. | .. | 0 | .. | .. | .. | 0 | 0 | .. | .. | 0 | 0 | .. |
| $13^h14^m$ | $13^h49^m$ | .. | 2 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| $13^h49^m$ | $14^h40^m$ | .. | 0 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |

[a] Rainfall, night previous, 0.09 inch. Time of cessation, between 3.25 and 3.55 a. m.

TABLE XI.—*Summary of records of ascospore-trap collections in 1913 at West Chester, Pa.*

| Date. | Rainfall. | Number of traps examined. | Time from cessation of rain to— | | Number of perithecia expelling ascospores between times stated. |
|---|---|---|---|---|---|
| | | | Replacing of slides. | Collection of slides. | |
| | *Inches.* | | | | |
| Aug. 27.... | 0.12 | 19 | .......... | $0^m$ to $18^m$ | 0 |
| | | 19 | $0^m$ to $18^m$ | $1^h22^m$ to $1^h27^m$ | 1 |
| | | 14 | .......... | $0^m$ to $15^m$ | 1 |
| Do.... | .055 | 14 | $0^m$ to $15^m$ | $45^m$ to $1^h$ | 360 |
| | | 14 | $45^m$ to $1^h$ | $14^h$ (Aug. 28) | 227 |
| | | 19 | .......... | $2^h6^m$ to $2^h37^m$ | 1,047+ |
| | | 19 | $2^h6^m$ to $2^h37^m$ | $3^h19^m$ to $3^h54^m$ | 53 |
| Sept. 18.... | .26 | 19 | $3^h19^m$ to $3^h54^m$ | $4^h20^m$ to $4^h38^m$ | 9 |
| | | 19 | $4^h20^m$ to $4^h38^m$ | $5^h47^m$ to $7^h19^m$ | 35 |
| | | 9 | $5^h47^m$ to $6^h37^m$ | $12^h25^m$ to $13^h10^m$ | 1 |
| | | 8 | .......... | $1^h48^m$ to $2^h4^m$ | 236 |
| Sept. 19.... | .68 | 10 | .......... | $3^h35^m$ to $5^h30^m$ | 219 |
| | | 8 | $1^h48^m$ to $2^h4^m$ | $3^h35^m$ to $5^h30^m$ | 15 |
| | | 17 | $3^h35^m$ to $5^h30^m$ | $12^h10^m$ to $13^h$ | 46 |
| | | 19 | .......... | $2^h48^m$ to $3^h10^m$ | 875+ |
| | | 19 | $2^h48^m$ to $3^h10^m$ | $4^h5^m$ to $4^h27^m$ | 433 |
| | | 19 | $4^h5^m$ to $4^h27^m$ | $5^h23^m$ to $5^h41^m$ | 130 |
| | | 19 | $5^h23^m$ to $5^h41^m$ | $6^h12^m$ to $6^h33^m$ | 28 |
| Sept. 20.... | .09 | 14 | $6^h12^m$ to $6^h33^m$ | $6^h53^m$ to $7^h11^m$ | 10 |
| | | 7 | $6^h53^m$ to $7^h11^m$ | $7^h35^m$ to $7^h47^m$ | 5 |
| | | 18 | $7^h35^m$ to $7^h47^m$ | $10^h34^m$ to $11^h31^m$ | 12 |
| | | 6 | $10^h34^m$ to $11^h31^m$ | $13^h14^m$ to $13^h38^m$ | 9 |
| | | 1 | $13^h14^m$ | $13^h49^m$ | 2 |
| | | 21 | .......... | $1^h40^m$ to $2^h$ | 2,494+ |
| Sept. 21.... | .43 | 21 | $1^h40^m$ to $2^h$ | $3^h6^m$ to $3^h22^m$ | 211 |
| | | 19 | $3^h6^m$ to $3^h22^m$ | $5^h28^m$ to $5^h36^m$ | 0 |
| | | 11 | .......... | $33^m$ to $41^m$ | 1,199+ |
| Do.... | .10 | 11 | $33^m$ to $41^m$ | $53^m$ to $1^h1^m$ | 14 |
| | | 6 | $53^m$ to $1^h$ | $1^h31^m$ | 0 |
| | | 21 | .......... | $5^h10^m$ to $5^h30^m$ | 577+ |
| | | 21 | $5^h10^m$ to $5^h30^m$ | $6^h31^m$ to $6^h51^m$ | 27 |
| Sept. 22.... | .73 | 21 | $6^h31^m$ to $6^h51^m$ | $8^h6^m$ to $8^h25^m$ | 12 |
| | | 21 | $8^h6^m$ to $8^h25^m$ | $9^h35^m$ to $11^h11^m$ | 3 |
| | | 2 | $10^h39^m$ to $11^h2^m$ | $14^h12^m$ to $14^h16^m$ | 6 |
| | | 10 | .......... | $1^h3^m$ to $1^h11^m$ | 618 |
| Oct. 20.... | .85 | 3 | $1^h3^m$ to $1^h11^m$ | $1^h16^m$ to $1^h21^m$ | 82 |
| | | [a] 4 | .......... | $12^m$ to $27^m$ | 245 |
| | | 3 | $12^m$ to $27^m$ | $22^m$ to $31^m$ | 303 |
| | | 4 | $20^m$ to $31^m$ | $37^m$ to $43^m$ | 387 |
| | | 4 | $37^m$ to $43^m$ | $45^m$ to $54^m$ | 379 |
| Do.... | .01 | 4 | $45^m$ to $54^m$ | $57^m$ to $1^h9^m$ | 390 |
| | | 4 | $57^m$ to $1^h9^m$ | $1^h12^m$ to $1^h26^m$ | 262 |
| | | 4 | $1^h12^m$ to $1^h26^m$ | $1^h28^m$ to $1^h46^m$ | 152 |
| | | 4 | $1^h28^m$ to $1^h46^m$ | $1^h55^m$ to $2^h9^m$ | 37 |
| | | 4 | $1^h50^m$ to $2^h9^m$ | $5^h21^m$ to $5^h54^m$ | 2 |

[a] This summary includes the records of traps Nos. 58, 59, 72, and 74 only.

The data secured relative to the duration of ascospore expulsion after certain rains are given in a summarized form in Table XI. As will be seen, all of these, except the results obtained on October 20, bear reference to rains occurring during the progress of exposure-plate tests. Although the exact time of cessation of rain is a very important point, in the cases of September 18, 19, 20, and 22 it could not be more accurately determined, because the rains all ceased in the night. A comparison of the figures presented in these tables with the results obtained in the exposure-plate tests will show a close interrelation.

On October 20 another opportunity was offered to obtain data relative to duration of spore expulsion. By selecting only a few traps and changing the slides at much shorter intervals more in detail was learned in regard to the activity of the perithecia (Table XI).

Since the point had been often suggested that these ascospore-trap tests might not yield results typical of natural conditions because of the protection from drying afforded by the glass suspended over the bark and that because of this spore expulsion was greatly prolonged, occasion was taken on October 20 to determine the validity of this contention.

About two hours after the cessation of rain, when expulsion had apparently ceased under most of the ascospore traps that were being tested (Table XI), a number of clean slides were placed at random over other areas of perithecia-bearing bark which appeared still to be damp, to determine whether or not perithecia on bark unprotected by glass slides were expelling spores at this time. Owing to the high south wind and occasional sunshine, such promising areas of bark were found only on the north side of trunks, either where loosened bark about a bad lesion had become soaked or in locations more or less protected by sprouts or by stumps from the drying action of the wind. The slides were held in place by a cord tied around the trunk, and care was taken to prevent contact with the papillæ. The results obtained from these 22 test traps are given in Table XII.

TABLE XII.—*Record of test traps on October 20, 1913, at West Chester, Pa.*[a]

| Trap No. | Time of placing slide. | Time of collection. | Results of examination. |
|---|---|---|---|
| 1 | 11.20 a. m. | 1.30 to 2.00 p. m. | 0. |
| 2 | 11.25 a. m. | 1.30 to 2.00 p. m. | 1 faint spot. |
| 3 | 11.27 a. m. | 1.30 to 2.00 p. m. | 0. |
| 4 | 11.27 a. m. | 1.30 to 2.00 p. m. | 0. |
| 5 | 11.32 a. m. | 1.30 to 2.00 p. m. | 0. |
| 6 | 11.35 a. m. | 1.30 to 2.00 p. m. | 0. |
| 7 | 11.35 a. m. | 1.30 to 2.00 p. m. | 1 light spot. |
| 8 | 11.39 a. m. | 1.30 to 2.00 p. m. | 0. |
| 10 | 11.34 a. m. | 1.30 to 2.00 p. m. | 0. |
| 11 | 11.47 a. m. | 1.30 to 2.00 p. m. | 0. |
| 12 | 11.47 a. m. | 1.30 to 2.00 p. m. | 0. |
| 14 | 11.55 a. m. | 1.30 to 2.00 p. m. | 0. |
| 15 | 12.00 to 12.04 p. m. | 1.30 to 2.00 p. m. | 1 faint spot. |
| 16 | 12.00 to 12.04 p. m. | 1.30 to 2.00 p. m. | 0. |
| 17 | 12.00 to 12.04 p. m. | 1.30 to 2.00 p. m. | 0. |
| 18 | 12.00 to 12.04 p. m. | 1.30 to 2.00 p. m. | 0. |
| 19 | 12.00 to 12.04 p. m. | 1.30 to 2.00 p. m. | 0. |
| 20 | 12.00 to 12.04 p. m. | 1.30 to 2.00 p. m. | 3 spots; 1 rather heavy. |
| 21 | 12.00 to 12.04 p. m. | 1.30 to 2.00 p. m. | 0. |
| 22 | 12.17 p. m. | 1.30 to 2.00 p. m. | 1 light spot. |

[a] Rainfall, 0.86 inch. Time of cessation, 9.09 a. m. Wind, high SSW.

SUMMARY OF ASCOSPORE-TRAP TESTS

From the standpoint of wind dissemination, the all-important feature proved beyond doubt by these tests is that in every case where ascospore expulsion occurred at all it continued for a time after the cessation of the rain, thus insuring a supply of spores in the air.

A glance over these results shows that in a general way the volume of ascospore expulsion, as measured by the character and number of spots on the slides, is greatest during or shortly after the rain and decreases more or less uniformly as the bark dries. On August 27 the rains were of the thunderstorm type, being of very short duration, and consequently the perithecia had hardly been wet for a sufficient length of time when the rain ceased. The greatest volume of expulsion occurred, therefore, a little later, evidently between 15 minutes and 1 hour after the rain had ceased.

With the exception of the rain in the afternoon of September 21, the tests of September 18, 19, 20, 21, and 22 are rather unsatisfactory, since no records could be obtained until some time after the rain had ceased. The summary for these dates (Table XI) shows that the maximum volume of spore expulsion had occurred before the first collections were made, and whether the climax occurred during the rain or shortly afterwards can not be stated. In the case of the afternoon rain of September 21 very evidently the maximum volume of spore expulsion took place before 33 minutes had elapsed after the cessation of the rain.

On October 20, after the bark had been thoroughly saturated by a rain in the night, the greatest volume of expulsion occurred within one hour after the rain. Two hours and nine minutes later a light rain of 58 minutes' duration began, and the results secured after this shower (Table XI) show that in three traps tested the greatest volume of expulsion occurred, not during the rain, but after 22 to 43 minutes had elapsed since its cessation.

As to the rate of subsidence of ascospore expulsion after the rains, Table XI shows a marked contrast between the results obtained on different dates. This has been mentioned in the discussion of the exposure plates and the relation of the subsidence of ascospore expulsion to weather conditions. In the cases of September 18, 19, 20, and 22 the duration of expulsion is seen to have been prolonged after the rains, and in all cases except September 19 the data show that the rate of subsidence was very gradual. Except for the last three hours of the duration of expulsion on September 22, fog or cloudiness and low wind prevailed, and the weather conditions were not favorable to rapid drying of the bark.

After the rains of September 21 and October 20 the rate of subsidence of ascospore expulsion was relatively abrupt and rapid, and its duration was comparatively short, especially after the second rain on September 21. Here, again, the relation of duration of expulsion to rapidity of drying of the bark is shown, since the rains on these dates were followed by brisk winds, and, except for the second rain of September 21, by rapid clearing and sunshine. Such weather conditions were, of course, very conducive to the rapid drying of the bark.

The maximum duration of ascospore expulsion as determined by these tests after each of these rains is shown in Table XIII. In considering these data the weather conditions just described should be borne in mind.

TABLE XIII.—*Maximum duration of ascospore expulsion after the cessation of rain, as determined by the examination of slides in ascospore traps at West Chester, Pa., in 1913*

| Date. | Rainfall. | Maximum duration of spore expulsion after rain. | Date. | Rainfall. | Maximum duration of spore expulsion after rain. |
|---|---|---|---|---|---|
| | *Inches.* | *H. m.* | | *Inches.* | *H. m.* |
| Aug. 27 | 0.175 | 45 | Sept. 21 | 0.43 | 1 58 |
| Sept. 18 | .26 | 6 15 | 21 | .10 | 40 |
| 19 | .68 | 5 27 | 22 | .73 | 11 2 |
| 20 | .09 | 13 14 | Oct. 20 | .86 | 3 8 |

It should be mentioned in this connection that the figures given in the Table XIII were secured in all cases, except that of October 20, from bark that had been protected continuously by the trap slide from the drying action of the wind, and it is possible that under such conditions the duration of expulsion may be slightly prolonged. But the data relative to the maximum duration of expulsion on October 20 were secured from bark previously unprotected by slides, since seven perithecia in five exposed areas were found to be expelling spores after expulsion had ceased in all but one area protected by the ascospore traps (Table XII). These tests prove beyond doubt that under natural conditions certain exposed areas of diseased bark do remain wet enough to cause spore expulsion fully as long as the particular areas protected by the ascospore traps. Of course, such areas would usually be in locations more or less protected from the wind or sun; but, nevertheless, they would continue to act as a source of spores for wind dissemination as long as any expulsion was in progress.

The direct bearing of the results of these ascospore-trap tests upon the results obtained in the exposure plates has been brought out in the discussion of the latter topic.

## ASPIRATOR TESTS

It has already been brought out in the historical introduction that previous analyses of air by the aspirator method under natural conditions in the field during dry weather failed to show the presence of spores of the chestnut-blight fungus (2). Positive results were obtained, however, under artificial conditions in the field, and it seems probable that failure to detect spores under natural conditions was due to the fact that most of the analyses were made during dry weather. If positive results were obtained following periods of rain, that fact was not brought out in the discussion (2). In order to obtain definite information on this point, the aspirator tests reported in the following pages were made so as to include the filtration of air immediately following periods of rain, as well as during the intervening dry weather.

### METHOD OF MAKING THE ANALYSIS

The apparatus used in this series of tests consisted of a 4-liter aspirator bottle set on a level stump near the center of the field (fig. 1). The nearest trees were 15 feet north, 19 feet east, and 33 feet west, and the

nearest lesion was on a branch 13 feet to the north. The standard sugar-tube method of making a quantitative bacteriological analysis of air was employed. The bottle was refilled with 4 liters of water at intervals of 20 or 30 minutes, thus making the aspiration practically continuous. One sugar tube was generally used each day, and the quantity of air drawn through each tube averaged 58 liters, with a maximum of 96 liters. The medium employed was a 3 per cent dextrose agar, with a reaction of +10. Ten plates were poured for each test and were incubated and the colonies counted in the same way as those in the experiments with the water spore traps (p. 520).

TABLE XIV.—*Summary of results of aspirator tests in 1913 at West Chester, Pa.*

| Sugar tube No. | Date of aspiration. | Rainfall.[a] | Quantity of air represented. | Number of bacteria and yeasts per liter. | Total number of fungi per liter. | Number of spores of Endothia parasitica per liter. | Number of species of fungi. |
|---|---|---|---|---|---|---|---|
| | | *Inches.* | *Liters.* | | | | |
| 1 | Aug. 19 | 0.40 | 12 | 26.42 | 4.16 | 0 | 4 |
| 2 | Aug. 20 | 0 | 28 | 3.21 | 4.28 | 0 | 9 |
| 3 | Aug. 21 | 0 | 36 | .83 | 3.05 | 0 | 9 |
| 4 | Aug. 22 | 0 | 48 | 2.29 | 5.21 | 0 | 7 |
| 5 | Aug. 23 | .25 | 68 | 1.47 | 17.65 | 0 | 3 |
| 6 | Aug. 24 | 0 | 56 | 2.86 | 11.07 | 0 | 5 |
| 7 | Aug. 25 | 0 | 56 | 3.96 | 3.96 | .35 | 7 |
| 8 | Aug. 26 | 0 | 56 | 4.64 | 8.93 | 0 | 10 |
| 9 | Aug. 27 | .175 | 47 | 2.12 | 8.3 | 0 | 11 |
| 10 | ....do | ........ | 12 | 3.75 | 5.0 | .42 | 9 |
| 11 | Aug. 28 | 0 | 68 | 1.35 | 4.04 | 0 | 12 |
| 12 | Aug. 29 | ........ | 40 | 2.5 | 5.875 | 0 | 9 |
| 13 | Aug. 30 | 1.10 | 60 | 1.25 | 3.91 | 0 | 7 |
| 14 | Aug. 31 | 0 | 56 | 1.16 | 7.67 | 0 | 10 |
| 15 | Sept. 1 | 0 | 92 | 1.25 | 38.37 | 0 | 11 |
| 16 | Sept. 2 | 0 | 72 | 1.18 | 1.87 | 0 | 8 |
| 17 | Sept. 3 | 0 | 72 | 1.32 | 7.91 | 0 | 12 |
| 18 | Sept. 4 | 0 | 56 | 1.78 | 36.60 | 0 | 11 |
| 19 | Sept. 5 | 0 | 68 | 1.69 | 20.00 | 0 | 11 |
| 20 | ....do | 0 | 4 | 16.78 | 6.25 | 0 | 7 |
| 21 | Sept. 6 | 0 | 76 | 1.12 | 8.09 | 0 | 12 |
| 22 | Sept. 7 | ........ | 64 | 4.61 | 3.90 | 0 | 13 |
| 23 | Sept. 7 and 8 | .37 | 12 | 6.25 | 8.33 | 0 | 10 |
| 24 | Sept. 8 | ........ | 76 | .39 | 4.86 | 0 | 10 |
| 25 | Sept. 9 | 0 | 60 | .16 | 1.41 | 0 | 5 |
| 26 | Sept. 10 | 0 | 76 | 2.17 | 6.90 | 0 | 10 |
| 27 | Sept. 11 | 0 | 72 | ........ | 1.94 | 0 | 7 |
| 28 | Sept. 12 | ........ | 80 | 1.50 | 2.58 | 0 | ........ |
| 29 | Sept. 13 | .095 | 68 | .59 | 1.91 | 0 | 9 |
| 30 | Sept. 14 | 0 | 72 | .14 | 1.11 | 0 | 6 |
| 31 | Sept. 15 | 0 | 72 | .62 | 1.18 | 0 | 10 |
| 32 | Sept. 16 | 0 | 72 | .41 | 1.25 | 0 | 6 |
| 33 | Sept. 17 | ........ | 80 | 1.00 | 1.12 | 0 | 8 |
| 34 | Sept. 18 | .26 | 52 | 7.59 | 14.23 | .192 | 8 |
| 35 | ....do | ........ | 64 | .15 | 5.94 | 0 | 14 |
| 36 | Sept. 19 | .68 | 96 | 1.04 | 4.27 | 0 | 10 |
| 37 | Sept. 20 | .09 | 76 | .59 | 1.31 | 0 | 9 |
| 38 | Sept. 21 | .43 | 40 | 1.50 | 4.50 | .125 | 8 |
| 39 | ....do | .10 | 28 | 1.52 | 6.25 | .089 | 10 |
| 40 | Sept. 22 | .73 | 52 | 2.30 | 1.82 | 0 | 6 |
| 41 | Sept. 23 | 0 | 80 | .875 | 1.31 | 0 | ........ |
| | Average | ........ | 57.9 | 2.91 | 7.03 | ........ | 8.8 |

[a] All rains occurred during the night previous to the date of aspiration, except on Aug. 27.

DISCUSSION OF RESULTS OF ASPIRATOR TESTS

The results obtained from these tests are presented in Table XIV. The average number of bacteria per liter of air was 2.91, while the number of fungi per liter averaged 7.03. The number of fungus species represented in the cultures ranged from 3 to 14.

In only five instances did any colonies of the chestnut-blight fungus appear in culture, and the number of spores per liter was never large. It is not impossible that the small numbers of spores of *Endothia parasitica* obtained may be due to the effect of sunlight, for in those instances where the rains were followed by fair weather the aspirator was exposed to the direct rays of the sun for a part of the day. This may also be the explanation of the fact that no spores of *E. parasitica* were obtained after some of the rains when ascospore-trap collections made it certain that expulsion was taking place, notably those of September 7 and 8 and September 21 and 22 (Table XV). Unfortunately there are no published investigations which give any information on the effect of sunlight on ascospores of the chestnut-blight fungus.

TABLE XV.—*Relation of aspiration tests to rainfall in 1913 at West Chester, Pa.*

| Date of rain. | Rainfall. | Date of aspiration. | Quantity of air tested. | Number of spores of Endothia parasitica to 10 liters of air. | Results with exposure plates. |
|---|---|---|---|---|---|
| | *Inches.* | | *Liters.* | | |
| Aug. 27 | 0.175 | Aug. 27 | 12 | 4.2 | + |
| 29–30 | 1.10 | 30 | 60 | 0 | − |
| Sept. 7–8 | .37 | Sept. 7–8 | 12 | 0 | + |
| 12–13 | .095 | 13 | 68 | 0 | − |
| 17–18 | .26 | 18 | 52 | 1.92 | + |
| 18–19 | .68 | 19 | 96 | 0 | + |
| 19–20 | .09 | 20 | 76 | 0 | + |
| 20–21 | .43 | 21 | 40 | 1.25 | + |
| 21 | .10 | 21 | 28 | .89 | + |
| 21–22 | .73 | 22 | 52 | 0 | + |

The chief explanation of the small number of spores of *Endothia parasitica* to the liter is to be found in the small amount of air drawn through each tube. While this averaged 38 liters for those tubes yielding positive results, only a few liters were drawn through the tube in the several hours during which copious expulsion of ascospores took place. The figures given in the tables are therefore smaller than the actual number of spores per liter during the period of copious expulsion. In view of these facts, Tables XIV and XV do not represent the true number of ascospores present in the air during the time of their actual prevalence, since the period of aspiration included many hours when they were not prevalent, as shown by the exposure-plate tests.

The rate of development of the colonies of the chestnut-blight fungus showed that they all originated from ascospores and none from pycnospores (5).

The spores obtained from sugar tube No. 7 two days after a rain may have been stray spores similar to those obtained in several exposure plates.

The aspirator tests do not appear to have given as reliable results as the exposure-plate method, since it may be noted from Table XV that negative results were obtained on certain days when the exposure plates showed that ascospores were prevalent. The importance, however, of the aspirator tests lies in the fact that ascospores were obtained under perfectly natural conditions in the field at a distance of 13 feet from the nearest lesion and that they were obtained at times when ascospore expulsion was taking place.

## WATER SPORE-TRAP TESTS

The use of water spore traps for testing the transport of spores of the chestnut-blight fungus by the wind was the outcome of our attempts to use the method of Burrill and Barrett (3) in their study of the wind dissemination of *Diplodia zeae*. First, substituting a funnel for the glass plates employed by the writers just cited, an attempt was made to find some mixture which could be applied to the inner surface of the funnel and which would fulfill the necessary requirements, as follows:

1. The mixture must contain no substances toxic to spores of the chestnut-blight fungus.
2. It must spread readily and adhere to a glass surface.
3. It must be sticky, so as to retain the spores which lodge upon the surface, which is coated with it.
4. It must retain its sticky character at least 24 hours under field conditions.
5. It must be readily soluble in water.

Glycerin of various percentages was tried alone, as well as in combination with various quantities of gum arabic or gelatin, but in all cases the mixtures either dried too soon or did not spread well on a glass surface.

The fact that pycnospores do not germinate in water (4) suggested the substitution of dishes of sterile water for the funnels. The first idea was that analyses of the water from these dishes exposed in the field under natural conditions could be made at intervals of some days and would reveal the presence of pycnospores if they had been carried by the wind. Experience in the field, however, proved that the method was also well adapted to the study of ascospore dissemination.

### DESCRIPTION OF THE WATER SPORE TRAPS

A water spore trap consisted of a crystallizing dish 5 cm. deep and 10 to 12 cm. in diameter, into which sterile water was introduced. The dishes were wrapped in paper and sterilized in the laboratory for transport to the field. Each dish was supported about 2 feet above the ground by a tripod of three small stakes driven into the ground. Ten-penny nails were driven into the ends of the stakes, whose ends were converged to make a support for the dish. The nails were held in proper position by a heavy cord attached to them and encircling the dish. By this means they were so firmly secured that they were never in danger of being blown out by the wind (Pl. LXIV, fig. 3). After placing a dish in its proper field location, 100 to 150 c. c. of sterile water were introduced. Water for this purpose was kept in stock in small Erlenmeyer flasks.

The dishes of water were exposed in the field in various selected locations and analyses made at certain intervals (fig. 3; also Pl. LXV, figs. 1 and 2).

### METHOD OF MAKING A TEST

At the end of an exposure period the contents of each dish were emptied into sterile flasks provided for the purpose and transported to the laboratory at the University of Pennsylvania, where the work of making an analysis was completed. They were then replaced with other sterile dishes and sterile water introduced as before.

For each water spore trap 15 to 20 plate cultures were employed, and these were made by introducing 0.1 to 0.5 c. c. of the water by means of a graduated 1 c. c. pipette into each Petri dish. In this way only 4 to 5 c. c. of the total water returned to the laboratory were used in each

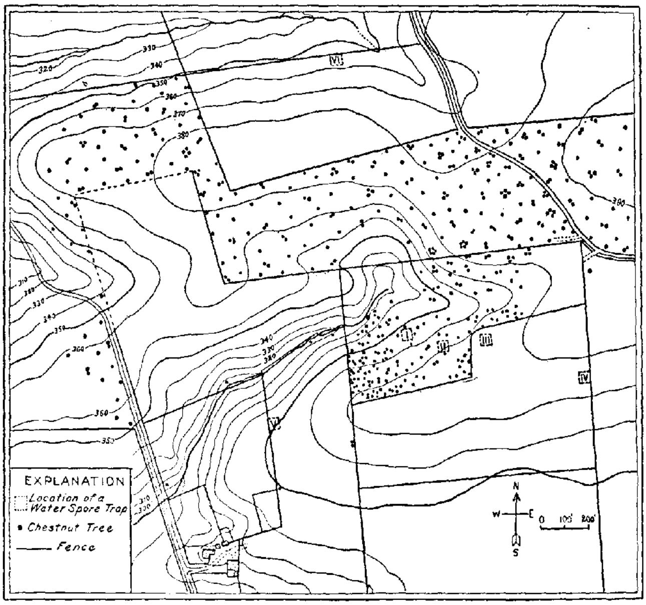


FIG. 3.—Map showing the location of water spore-trap stations Nos. I to VI. Stations I and II are in the chestnut coppice, the detailed composition of which is shown in figure 1; Stations III to V are at various distances from the same coppice; Station VI is to the north of a mixed chestnut and oak woodland.

test, but special pains were taken to secure a uniform suspension before the removal of the quantities used. Chestnut-bark agar was used for all of these analyses (see p. 496 for formula) since experience had proved that it was a poor medium for the growth of bacteria, which were always present in some quantity. In fact, the medium is so unfavorable for the development of ordinary bacteria that in most cases the colonies remained as minute specks during the period the plates were under observation and with proper dilution offered no hindrance to the development of colonies of *Endothia parasitica* and other fungi. All cultures were incubated as nearly as possible at 25° C., and the colonies of fungi suspected of being the chestnut-blight fungus were marked at the end of three days. The count was completed on the fifth day, and any uncertain colonies

were transferred to 3 per cent dextrose agar for further study. In general, it may be said that transfers were not necessary, for the colonies of *E. parasitica* are very characteristic on chestnut-bark agar at the end of five days, if they have had sufficient room in which to develop. It was only in the case of plates badly crowded with other fungi that such transfers were necessary.

## RESULTS AND DISCUSSION OF TESTS

The water spore traps were exposed in or near the same plot of badly diseased chestnut trees at West Chester, Pa., which was employed for the exposure plates previously reported. Six different stations were selected for the location of water spore traps at distances varying from 15 to 389 feet from the nearest blight lesions, although Station V was 404 feet from the nearest probable source of spores. More detailed information in regard to these stations is given in Table XVI.

TABLE XVI.—*Relation of water spore-trap stations to diseased chestnut trees in 1913 at West Chester, Pa.*

| Trap station No. | Distance and direction from station to nearest lesions. | Position of station with reference to diseased chestnut trees. |
|---|---|---|
| I....... | 25 feet west and east........ | Surrounded by 15- to 18-year-old coppice. |
| II....... | 15 feet southwest; 19 feet north. | Do. |
| III....... | 50 feet north; 50 feet west.. | In cornfield 50 feet from coppice. |
| IV....... | 383 feet northwest......... | Across cornfield from coppice. |
| | 398 feet north.............. | Across open field from a single tall tree. |
| V....... | 237 feet northwest......... | Across pasture from a single tall tree. |
| | 265 feet south.............. | Do. |
| | 317 feet northeast.......... | Across cornfield from a single tall tree. |
| | 404 feet east............... | Across cornfield from coppice. |
| VI....... | 389 feet south.............. | Across hayfield from older forest. |

TABLE XVII.—*Summary of the tests with water spore traps in 1913, at West Chester, Pa.*

| Test No. | Trap station No. | Culture Nos. | Period of exposure. | Date of cultures. | Total number of fungous spores. | Number of spores of Endothia parasitica. |
|---|---|---|---|---|---|---|
| 1 | I | 4227–4234 | Aug. 30 to Sept. 4.. | Sept. 5.. | 11, 215 | 0 |
| 2 | II | 4235–4242 | .....do............ | ...do..... | 13, 868 | 0 |
| 3 | III | 4243–4250 | Aug. 31 to Sept. 4.. | ...do..... | 23, 239 | 0 |
| 4 | III | 4821–4835 | Sept. 4 to Sept. 8... | Sept. 10.. | 1, 236 | 0 |
| 5 | I | 4906–4920 | Sept. 4 to Sept. 13.. | Sept. 16.. | 6, 375 (?) | 0 |
| 6 | I | 4951–4960 | Sept. 13 to Sept. 18. | ...do..... | 10, 666 | 1, 749 |
| 7 | II | 4967–4976 | .....do............ | ...do..... | 10, 583 | 3, 507 |
| 8 | III | 4983–4992 | .....do............ | ...do..... | 7, 886 | 2, 113 |
| 9 | I | 5303–5312 | Oct. 13 to Oct. 20... | Oct. 21... | ............ | 0 |
| 10 | VI | 5313–5322 | Oct. 20, a. m.–p. m. | ...do..... | 1, 666 | 30 |
| 11 | IV | 5345–5354 | Oct. 19 to Oct. 22.. | Oct. 23.. | ............ | 0 |
| 12 | V | 5355–5364 | .....do............ | ...do..... | ............ | 0 |
| 13 | I | 5371–5380 | Oct. 22 to Oct. 27.. | Oct. 28... | 22, 139 | 378 |
| 14 | IV | 5381–5390 | .....do............ | ...do..... | 13, 148 | 380 |
| 15 | V | 5391–5400 | .....do............ | ...do..... | 4, 591 | 820 |
| 16 | VI | 5401–5410 | .....do............ | ...do..... | 113, 203 | 431 |
| 17 | I | 5411–5420 | Oct. 27 to Nov. 10.. | Nov. 11.. | Numerous. | 0 |
| 18 | V | 5421–5430 | .....do............ | ...do..... | Numerous. | 0 |
| 19 | VI | 5431–5440 | .....do............ | ...do..... | Numerous. | 0 |

The map (fig. 3) shows the location of the coppice growth and other chestnut trees than those used in the test, with the position of the exposure stations. The character of the diseased coppice growth is shown in Plate LXV, fig. 1, which shows a view taken from Station V. The older forest, which was the source of the ascospores for the traps exposed at Station VI, is shown in Plate LXV, fig. 2. The period from August 30 to November 11, 1913, was covered by the tests presented in Table XVII.

The time and amount of rainfall, and in some cases the wind direction, are necessary in interpreting the results. Table XVIII gives the rainfall for the time covered by the water spore-trap tests.

TABLE XVIII.—*Rainfall record for period covered by the water spore-trap tests in 1913 at West Chester, Pa.*

| Date of rain. | Rainfall. | Date of rain. | Rainfall. | Date of rain. | Rainfall. |
|---|---|---|---|---|---|
| | *Inches.* | | *Inches.* | | *Inches.* |
| Aug. 29 and 30 | 1.10 | Sept. 21 and 22 | 0.83 | Oct. 20........ | 0.08 |
| Sept. 7 and 8 | .37 | 30....... | .02 | 24........ | |
| 12 and 13 | .095 | Oct. 1......... | 1.12 | 25........ | 1.44 |
| 17 and 18 | .26 | 2......... | .12 | 26........ | |
| 18 and 19 | .68 | 3......... | .06 | Nov. 8 and 9.. | 1.00 |
| 19 and 20 | .09 | 11......... | .73 | | |
| 20 and 21 | .43 | 19......... | .86 | | |

Tests Nos. 1, 2, and 3 were started in the field after the rain of August 29 and 30, late in the day, and the traps were taken to the laboratory for analysis before the next rain. Judging from the results obtained from our exposure plates, no ascospores should have been present, and our failure to get any colonies of the chestnut-blight fungus in the test cultures suggests that during that period there was no wind dissemination of either pycnospores or ascospores. There was a small amount of rain during the period that traps 4 and 5 were exposed, but the analyses were not made until two and three days later. Considering the fact that ascospores germinate at once in water, the failure to get any colonies of the *Endothia parasitica* in these tests is not surprising and again points to the absence of pycnospores. Traps 6, 7, and 8 were removed from the field a few hours after the heavy rain of September 18 and 19, and the analyses gave a large number of colonies of the chestnut-blight fungus. It appears probable that the spores were caught during the few hours following the rain, since the cultures indicated the origin of the colonies from ascopores only (5). It should be noted from Tables XVII and XVIII that traps 9 to 12 were removed from the field just following periods of rain. The wind was blowing from the infected trees toward trap 10 only, and this was the only one in the series which yielded the blight fungus. Traps 13 to 16 were removed from the field shortly after the rainy period of October 24 to 26, and all yielded positive results, trap 16, located 389 feet from the nearest chestnut tree, giving 431 spores. The length of time after the rain when the tests were made and the direction of the wind are the possible explanation for the negative results for traps 17 to 19. Unfortunately no traps were exposed during the rainy periods of October 1 to 3 and October 11.

It is probable that the figures recorded for tests Nos. 6 to 8 and 13 to 16 represent the number of spores blown into the traps during the few hours

following the rain. This appears to be substantiated by negative results obtained during dry periods and by positive results obtained with exposure plates and slide traps just following a period of rain. The results are briefly summarized in Table XIX. It is interesting to note the number of viable ascospores of the chestnut-blight fungus that must have fallen on each square inch of water surface for the time represented. This information is presented in Table XX.

TABLE XIX.—*Summary of positive results obtained from water spore traps in 1913 at West Chester, Pa.*

| Station. | Number of tests represented. | Distance of station from nearest lesion. | Total number of spores of Endothia parasitica caught, as determined by cultures. |
|---|---|---|---|
| | | *Feet.* | |
| I | 2 | 25 | 2,136 |
| II | 1 | 15 | 3,507 |
| III | 1 | 50 | 2,113 |
| IV | 1 | 383 | 380 |
| V | 1 | 237 | 820 |
| VI | 2 | 389 | 461 |

TABLE XX.—*Number of ascospores of Endothia parasitica falling on each square inch of water surface at various distances in 1913 at West Chester, Pa.*

| Test No. | Surface area of water trap. | Number of spores of Endothia parasitica falling on each square inch of water surface. | Distance to nearest lesion. | Test No. | Surface area of water trap. | Number of spores of Endothia parasitica falling on each square inch of water surface. | Distance to nearest lesion. |
|---|---|---|---|---|---|---|---|
| | *Sq. inches.* | | *Feet.* | | *Sq. inches.* | | *Feet.* |
| 6 | 12.5 | 139 | 25 | 14 | 16.5 | 23 | 383 |
| 7 | 12.5 | 280 | 15 | 15 | 16.5 | 50 | 237 |
| 8 | 12.5 | 169 | 50 | 16 | 16.5 | 26 | 389 |
| 13 | 16.5 | 23 | 25 | | | | |

The large number of spores of *Endothia parasitica* falling on each square inch of surface for a single rainy period certainly emphasizes the fact that healthy trees in the vicinity of badly diseased ones have innumerable opportunities to become infected by wind-borne spores.

It should be mentioned in this discussion of the results obtained by the water spore traps that there are some possibilities of error. It might be claimed that the spores found in the water traps were carried by birds or insects. This, however, appears exceedingly improbable. The cultures always indicated ascospores and tests have shown that birds are carriers of pycnospores only (8). The position of the traps was such as to reduce the insect visitors to a minimum. Insects tested as carriers of the chestnut-blight fungus yielded both pycnospores and ascospores, but the former were very much more abundant (17). Besides, it was rare that any insects were found in the exposure dishes. Furthermore, spores were present in the traps only at periods following rains when other tests had indicated their prevalence.

## CONCLUSIONS

(1) As a result of 756 exposure plates made in or near the badly diseased chestnut coppice at West Chester, Pa., it can be definitely stated that ascospores of *Endothia parasitica* (Murr.) And. are prevalent in the air and after expulsion are carried for varying distances from their source.

(2) As shown by the same exposure plates, the period of prevalence of ascospores varies with the conditions following the cessation of rains; when there is a rapid drying of the bark, this period is short, but when drying is retarded, this period is correspondingly extended. The tests indicate a general prevalence of ascospores within the first 5 hours following the cessation of rains, with less abundance during later hours. The longest period for our entire series was 14 hours.

(3) During periods of dry weather ascospores, although not generally prevalent, may occasionally be detected by the exposure-plate method. These are apparently stray ascospores expelled during some previous period of rain and now loosened from lodgment on some near-by objects.

(4) In and near badly diseased chestnut groves or forests the number of ascospores falling on each square foot of exposed surface following a period of rain, as indicated by exposure plates, is very large and is sufficient to offer abundant opportunity for new infections.

(5) Ascospores are forcibly expelled in large numbers from the perithecia during and after each warm rain in case the amount is sufficient to soak up the pustules. Following a dry period a rain of 0.18 to 0.25 inch has been observed to cause copious expulsion of ascospores, while rains of 0.01 to 0.10 inch, if immediately preceded by a copious rainfall, have been sufficient to cause the resumption of spore expulsion.

(6) As determined by the ascospore traps, the duration of expulsion depends on the rapidity with which the bark dries and only continues when the stromata are moist. Under natural conditions in the field the period of expulsion for eight rains varied from 45 minutes to 13 hours and 14 minutes.

(7) In some cases at least the maximum of ascospore expulsion occurs after the cessation of rain.

(8) The fact that the period of ascospore expulsion as determined by the ascospore traps coincides in general with the period during which spores were obtained by exposure plates points to these forcibly expelled spores as the ones prevalent following periods of rain. This is definitely substantiated by the development of colonies in the exposure plates from ascospores only.

(9) It is possible to determine the presence of ascospores of the chestnut-blight fungus in the air under natural conditions in the field by the standard aspirator method of bacteriological analysis. By this method positive results were obtained following four different rainy periods, but only when the period of aspiration included a period of copious ascospore expulsion.

(10) By the use of water spore traps stationed at varying distances from diseased trees it was possible to determine that ascospores are prevalent in the air and fall upon exposed surfaces in considerable numbers, the number diminishing with the distance from the source of supply.

(11) By making possible long exposures the water spore traps offered some advantages over the exposure-plate and aspirator methods. The presence of spores of the chestnut-blight fungus, however, was never shown by this method unless the period of exposure included a period of ascospore expulsion.

(12) The failure to obtain colonies of the *Endothia parasitica* from the water spore traps exposed during dry periods, as well as the fact that only ascospore colonies were indicated in the aspirator and exposure-plate tests, points to the conclusion that pycnospores are not generally prevalent in the air at any time. If present they certainly would be detected by the prolonged exposure of water spore traps.

(13) The time immediately following a rain, when the bark is still moist, would appear to be a favorable one for new infections, since the supply of moisture would offer opportunity for germination of spores. It is a noteworthy fact that it is only during this favorable period for germination that the dissemination of ascospores takes place.

(14) All of these experiments point to air and wind transport of the ascospores of the chestnut-blight fungus as one of the very important methods of dissemination and substantiate the conclusions of Rankin (15, 16) and Anderson (1, 2). It can now be said with absolute certainty that following each warm rain of any amount ascospores are carried away from diseased trees in large numbers. Since they have been obtained in large numbers at distances of 300 to 400 feet from the source of supply, the conclusion of the authors that they may be carried much greater distances is justified. During dry periods wind dissemination of ascospores does not occur at all or sinks to a very insignificant minimum.

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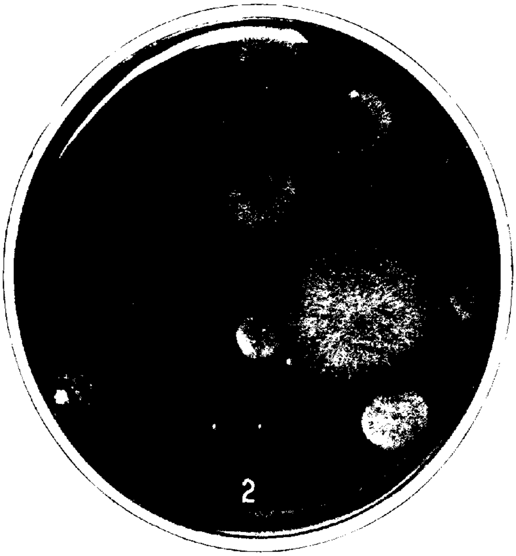
## PLATE LXIII

Fig. 1.—Petri-dish culture 5044 from 12 minutes' exposure of chestnut-bark agar, made on September 20, 1913, 2 hours and 8 minutes after the cessation of a rain, at station 51, located 27 feet from the nearest lesion.

Fig. 2.—Petri-dish culture 5041 from 16 minutes' exposure of chestnut-bark agar, made on September 20, 1913, 1 hour and 55 minutes after the cessation of a rain, at station 49, located 414 feet from the source of the spores. Ten of the twelve colonies are those of *Endothia parasitica*.


1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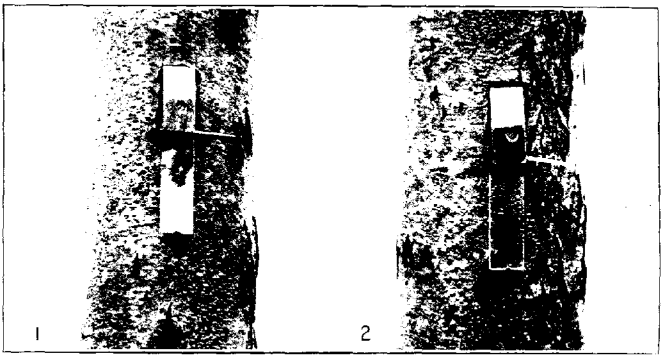

PLATE LXIV

Fig. 1.—Ascospore trap 51. This consists of a wooden bracket which supports an object slide over perithecial pustules.

Fig. 2.—Ascospore trap 52.

Fig. 3.—Water spore trap located at Station V. The trap consists of a crystallizing dish containing sterile water and is supported on a tripod.

PLATE LXV

Fig. 1.—View looking towards the coppice growth from water spore-trap Station V. The trees in the background at the right are at the end of the plot shown in text figure 1.

Fig. 2.—View of a mixed chestnut and oak grove taken from water spore-trap Station VI. This grove was the source of the spores of the blight fungus caught at Station VI.

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